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STUDIES ON THE PARATHYROID OF THE MOUSE*

2. SOME OBSERVATIONS UPON THE POST-NATAL CYTOLOGICAL DEVELOPMENT OF THE NORMAL GLAND, WITH PARTICULAR REFERENCE TO ITS MITOTIC ACTIVITY AND GOLGI ELEMENTS

By C. L. FOSTER, *Department of Biology, Middlesex Hospital, London, W.* 1

INTRODUCTION

In a previous paper (Foster, 1943*a*) an account was given of the cytological structure of the parathyroid gland of the adult mouse, without any very detailed description of its cytological development. The latter is described in the present paper.

MATERIAL AND METHODS

Glands from forty-six male or female mice varying from 1 to 131 days old were employed for studying the development of the various cell types. Twenty-two of these animals, ranging from 2 to 64 days old, were also used for the study of the Golgi elements, the parathyroids being impregnated by the Nassonov-Kolatschew method as previously described (Foster, 1943*a*). Forty-three glands from animals 6–131 days old were used for the study of mitotic activity. The animals were injected with colchicine (Foster, 1943*b*) and the glands were fixed in Champy's or Zenker's fluid. The staining methods used have been previously described (Foster, 1943*a*).

RESULTS

The development of the cell types

There was considerable variation in the degree of differentiation of glands at any given age, and even between those of the same animal. These variations did not interfere with the observation of developmental trends, provided that no attempt was made to equate degrees of differentiation with particular ages.

In young mice up to about 10 days old, the gland was compact with a uniform parenchyma of cells not markedly divided into lobules by connective tissue. At about 3 weeks after birth, when the animals were usually weaned, the gland had grown considerably and was now lobular, owing to an increase in connective tissue. This histological differentiation was accompanied by an increased vascularity, the larger vessels running in the connective tissue septa. In a few of the younger glands, irregularly shaped, elongated cells containing yellowish brown pigment granules were occasionally seen associated with the connective tissue around the larger blood-vessels. Erdheim (quoted by Gilmour, 1939) has reported perivascular pigmented cells in the parathyroid of infant and adult humans.

In a previous paper (Foster, 1943*a*) the following cell types in the adult parathyroid were described: (*a*) a 'chief' cell—now to be called a 'stem' cell; (*b*) a 'dark' cell; (*c*) a 'transitional' cell; (*d*) a 'clear' cell. Until the end of the first week after birth, the gland was composed entirely or almost entirely of stem cells. These cells varied somewhat in their nucleus/cytoplasm ratio but usually the nucleus was relatively large (Fig. 1 *a*, *b* and Fig. 3 *b*, *c*). The cytoplasm looked clear or contained pale, clouded areas and stained only faintly. The nuclei were multinucleolar. At the end of this stage (D_1 in Fig. 5) there began to appear smaller cells of a more oval shape, as if compressed between the other cells; they had all the characteristics of the dark cells of later stages of development. With increasing age the dark cells became more common and some, on account of their being of the same shape and order of size as stem cells, appeared to have originated directly from them. During the second and third weeks after birth the dark cells began to form a characteristic component of the gland, and the oval ones were commonly found in small groups as in the adult. This phase of development is indicated at D_2 in Fig. 5. As an example of the variation encountered, two 23-day-old litter mates may be cited. In one, the parathyroids showed frequent dark cells; one gland from the other had the same composition while the second gland was still composed predominantly of stem cells. Such disparities, however, did not affect the general conclusion that the dark cells appeared later than the stem cells and became increasingly common with age.

Both transitional and clear cell types first appeared between the 2nd and 3rd weeks after birth (see $(C+T)_1$, Fig. 5); they were observed in one animal of 18 days, but were not frequent in such young mice. These cells are illustrated in Fig. 2 *c*, *d* and *e*. The appearance and increase in number of the transitional and clear cells and the increase in the dark-cell population was accompanied by a

* Part of a thesis approved for the degree of Doctor of Philosophy in the University of London.

decrease in the number of stem cells, and the gland became more and more adult in appearance. The transformation was gradual, but by the end of the 5th week the glands were generally indistinguishable from the adult type ($(C+T)_2$, Fig. 5).

THE MITOTIC ACTIVITY OF THE DEVELOPING GLAND

An investigation of the mitotic activity of the gland was undertaken to discover: (1) whether the appearance of the oval dark cells in groups during the 2nd and 3rd weeks of post-natal development was accompanied by an increase in mitotic activity, and (2) whether cells other than stem cells underwent mitosis, as, for example, do the chromophil cells of the anterior pituitary (Pomerat, 1941; Kerr, 1943).

The colchicine injections were made at the same time in the morning in all animals so that the effects of any diurnal rhythm might be minimized. That the injections were effective in all cases, was demonstrated by the high frequency of mitotic figures in the oesophageal epithelium which was used as a check on the action of the drug. The trachea and larynx were always cut longitudinally in such a manner that the plane of section of the glands was the same in all instances.

The following data in the form of an average for each group of five sections from the region of maximum cross-sectional area, were obtained from every gland. First, the number (m) of mitoses per section (the bulk of the mitotic figures were metaphase stages, but prophase and other stages were included in the counts wherever they were observed); secondly, the area (a) of the section, measured in unit squares of a micrometer scale at a magnification of 350 diameters, and thirdly, the number of cells (n) per unit square at a magnification of 700 diameters—five values for each section. The mitotic frequencies were standardized by calculating the values of $\frac{m}{4an} \times 1000$ which gave the approximate number of mitoses per 1000 cells in the area of maximum cross-section. In this way a mitotic index was obtained, and the mean results for the various 5-day age groups are shown in Table 1. They are also expressed in the form of a histogram in Fig. 5. The data recorded include observations additional to those already reported upon (Foster, 1943*b*).

Table 1

Age groups (in days)	No. of glands in group	Average mitotic index (no. of mitoses per 1000 cells) $= \frac{m}{4an} \times 1000$
6–10	6	0·7
11–15	8	3·7
16–20	12	7·1
21–25	3	6·7
26–30	5	1·0
31–35	4	1·1
90*	4	0·1
131*	1	0·1

* Not included in Fig. 5.

It will be seen from the histogram (Fig. 5) that the mitotic activity during the 1st week after birth was very slight, but from about 10 days onward there was a rapid rise, and then a sharp falling off just before the end of the 4th week. The peak period occurred when the animals were about 3 weeks old. The mitotic index for mature animals is not shown in the histogram, but Table 1 shows that it was very low—0·1. No values were obtained for animals less than 6 days old owing to the difficulty of injection.

Most of the cells in division possessed cytoplasm of a type to suggest that they were either stem or perhaps clear cells. The interpretation of the cell types was often complicated by the fact that the spindle region occupied a large area of the cytoplasm and was itself stained. In such instances, the identification of the cell could only be based on the staining reaction of the peripheral cytoplasm—whether pale or dark. In regard to the distinction between stem cells and clear cells, it seemed reasonable to suppose, on account of the absence or infrequency of the latter type at the ages under consideration, that in immature glands the majority of the pale cells in division were of the stem type, since the enlargement of the nuclear area made it difficult to assess the nucleus/cytoplasm ratio. Undoubted stem cells were observed in early prophase, but only occasionally, since after 9½ hr. of colchicine treatment most of the nuclei had passed on to metaphase. Clear cells in prophase were not observed. In sections from 18-, 21- and 26-day-old glands, mitotic figures were occasionally seen in cells having the denser basiphil cytoplasm characteristic of dark cells. A prophase and a metaphase stage were noticed in cells of this type from a 26-day-old mouse. The impression obtained was that the divisions of dark cells were not numerous. Since colchicine arrests cell division at metaphase it was not possible to observe the telophase stages, which might have given some indication as to whether the products of the division of dark cells were themselves dark. That further dark cells were produced from the division of dark cells seemed very likely however, since these cells were frequently observed to occur in clusters. It is quite possible also, that the transitional cells were capable of division, but no reliance was placed on a transitional staining reaction in dividing cells for the reason already given.

A small number of glands from colchicine-injected animals, prepared to show the Golgi bodies, demonstrated that these structures were of no value in 'labelling' the cell types, since they underwent fragmentation during cell division. The striking effect of colchicine upon these bodies has already been reported upon (Foster, 1944).

THE DEVELOPMENT OF THE GOLGI BODIES

It became clear from an examination of the whole range of immature glands, that the Golgi bodies showed a continuous sequence of changes with age. In order to classify the varied configurations met with over a developmental period of approximately 9 weeks, the Golgi bodies were divided into three reasonably well-defined groups, but at the same time two transitional states were recognized in addition. The latter, as well as the three principal types, are shown in Fig. 5, where their age distribution is shown graphically. The three principal configurations observed were as follows:

The compact type

This can probably be regarded as an embryonic type. It was found to assume several forms, all characterized by their compactness and their close proximity to the nucleus. In glands from 2-day-old mice, for example, the Golgi bodies were in the form of small close networks of granulated threads (Fig. 1*a*, *b*). In 5-day-old glands they varied from nets of this type, to single, coiled, ring-like or branched rather thickened granulated strands—all these forms being closely related to the nuclear region. In two cases in 5-day-old glands, the Golgi bodies showed a precocious development to the 'unfolded' type described below. Much the same kinds of compact configuration were observed in 7- and 9-day-old glands. Golgi bodies of this general type were seen in the adult gland where they were associated with cells of the stem type (Fig. 3*e*).

The unfolded type

This type appeared to be based on a further elaboration of the preceding form. The Golgi bodies here were larger, occupying a greater proportion of the cytoplasm. Although they remained either in whole or in part in a juxtanuclear position, they were much more complex than the compact forms. Small numbers of the latter were observed in glands whose Golgi bodies were predominantly of the unfolded type. Generally speaking, however, the change from one form to the other involved the bulk of the cells. Some of the unfolded types encountered in 11-, 20-, 23-, 26- and 33-day-old glands are illustrated in Fig. 1 (*c*–*k*). It will be observed that there were configurations which could be regarded as elaborations of the compact network type (Fig. 1*i*, *j*), whereas others appeared to be derived from the various forms of the compact stranded type (Fig. 1*f*, *g*). The majority of the dark cells, which were becoming quite frequent at this age level, had the stranded type of Golgi body, which was often stretched out along one side of the nucleus (Fig. 1*g*). This arrangement closely resembled that observed in similar cells in the adult gland, except that the Golgi material had a more elaborate form in the dark cells of these immature glands. Sometimes dark cells possessed Golgi bodies of the compact type, as if they had arisen by a direct transformation of stem cells.

The varied type

This type so closely resembled that found in the glands of adult animals that little need be said about it here, except to point out that the variations in form characterizing it may be classified into three types: (1) granular strands closely associated with the nuclear region (Fig. 3*a*, *i*); (2) strands arranged in a more dispersed manner, extending into the cytoplasm away from the nuclear zone (Fig. 3*g*, *h*); and (3) either an ill-defined reticulum (Fig. 3*d*) or a series of scattered threads and granules (Fig. 3*f*); but in a characteristically remote position in regard to the nucleus in both instances.

The transitional types

These have already been mentioned in the introduction to this section. One type showed features of a character intermediate between the 'compact' and 'unfolded' types; the other was intermediate between the 'unfolded' and the 'varied' types.

DISCUSSION

In what follows an attempt is made to correlate under the following headings the observations that have been made: (1) the mitotic activity of the gland, and (2) the cytological development of the gland, and the cell lineages involved.

The mitotic activity of the gland

Inspection of the histogram (Fig. 5) shows that it has the same general form as that of a growth increment curve. Since, however, there was between the 1st and 3rd weeks after birth a rapid increase in the proportion of cells in division, there may have been some special stimulus in operation. It is known, for example, that oestrogens are capable of inducing mitosis in the anterior pituitary (Kerr, 1943) and that continuous injections of dextrose given intravenously result in increased mitoses of the *b*-cells of the pancreatic islets (Woerner, 1938). It has also been reported by Blumenthal (1940) that anterior pituitary extract greatly increases the mitotic activity of the rat adrenal cortex. There is thus some evidence to suggest that glandular tissues may respond, by increased cell division, to certain substances whose concentration in the body fluids may fluctuate. Both de Robertis (1941) and

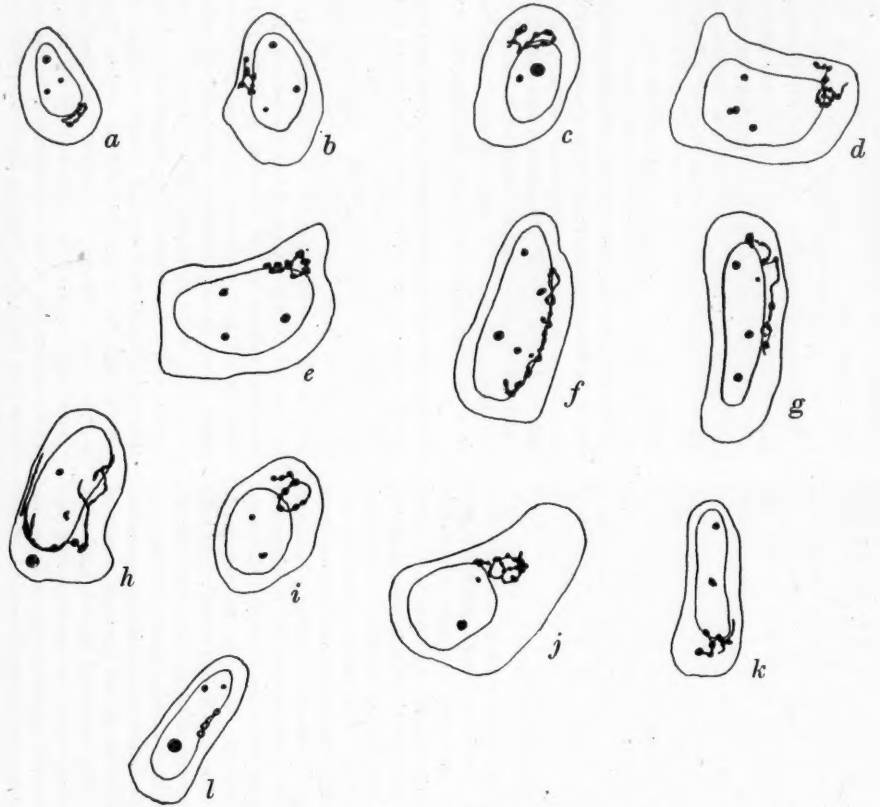

Fig. 1. Golgi bodies of cells from immature glands of various ages. *a*, 2-day male; *b*, 5-day male; *c*, 11-day male; *d*, 20-day female; *e*, 11-day male; *f*, 11-day female; *g*, 26-day animal; *h*, 20-day female; *i*, 23-day female; *j*, 23-day female; *k*, 33-day male; *l*, 9-day male. ×1000.

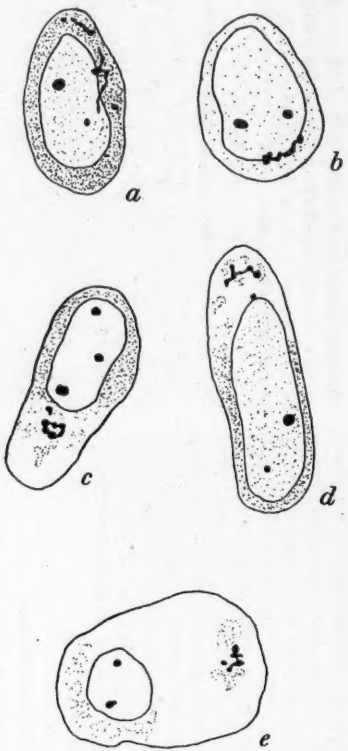

Fig. 2. Golgi bodies from the parathyroids of immature mice, showing Golgi bodies of the 'varied' type. *a*, Dark cell from 23-day male; *b*, stem cell from same; *c*, transitional cell from same; *d*, transitional cell from 25-day mouse; *e*, clear cell from same. ×1000.

mouse; *e*, clear cell from same. ×1000.

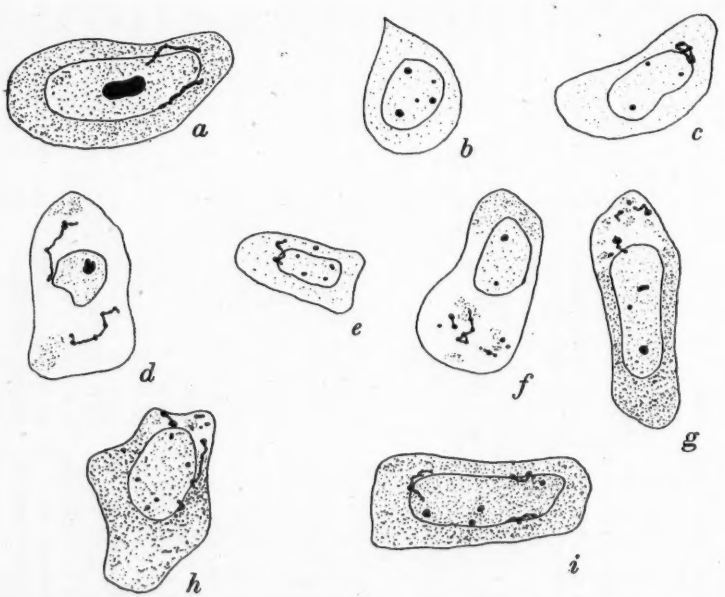

Fig. 3. Drawings of cells from an adult male gland (with the exception of *b* and *c*). Note the granular components of the Golgi body elements. *a*, Dark cell with Golgi material; *b*, stem cell from a 1-day male gland; *c*, stem cell from a 7-day male gland; *d*, a clear cell with dispersed Golgi material (note granular nature) and floccular areas in the cytoplasm; *e*, stem cell from adult gland with Golgi material capping the multinucleolate nucleus; *f*, clear cell with granular dispersed Golgi material and pale floccular cytoplasm; *g*, transitional cell showing clearer region in the cytoplasm above the nucleus (the Golgi material is largely dispersed into the outer cytoplasm); *h*, an early transitional cell with multinucleolate nucleus (part of the Golgi material is remote from the nuclear area and there is some 'rarefaction' of the cytoplasm in the region of the Golgi material); *i*, dark cell with the Golgi material in the form of several apparently separate, perinuclear granulated threads. ×1000.

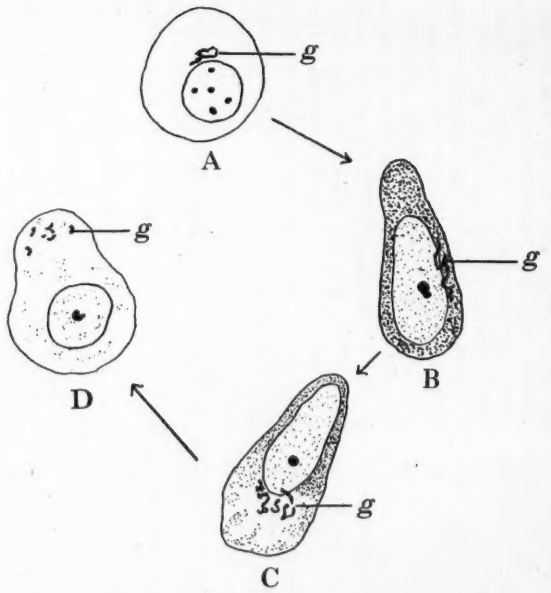

Fig. 4. *A*, stem cell; *B*, dark cell; *C*, transitional cell; *D*, clear cell; *g*, Golgi body. ×1000.

Figs. 1–4 are taken from camera lucida-drawings. The Golgi bodies were revealed by the Nassonov-Kolatschew technique. Figs. 1 and 2 are from unstained preparations; Figs. 3 and 4 from preparations stained by the Severinghaus method.

Sinclair (1941) have described an enlargement of the rat parathyroid gland following upon diets deficient in calcium, and Sinclair (1941) has also reported parathyroid hypertrophy during pregnancy. In the case of the young parathyroid, an alteration in the calcium level of the blood suggested itself as a likely stimulus to mitotic activity. During the period from birth to weaning, which, as the diagram shows, included the period of greatest mitotic activity, the most probable cause of any alteration in the calcium level would have been variations in the amount of this substance in the mother's milk. In this connexion there is the evidence of Horning (1942) for a decrease in the amount of mineral salts (and hence growth-rate was decreasing during the period when the mitotic index of the parathyroid gland was increasing. This fact suggested that an increased rate of cell division was not a general condition of all tissues and organs during this time.

For the purpose of comparing the cytological development of the gland with changes in its mitotic activity, the cytological history of the gland was divided into three phases, shown as *A*, *B* and *C* in Fig. 5. In phase *A*, during which the mitotic activity was low, the cellular components of the gland were almost exclusively of the stem type, and in phase *B*, when the mitotic activity showed a rapid increase to a peak value, the dark cells began

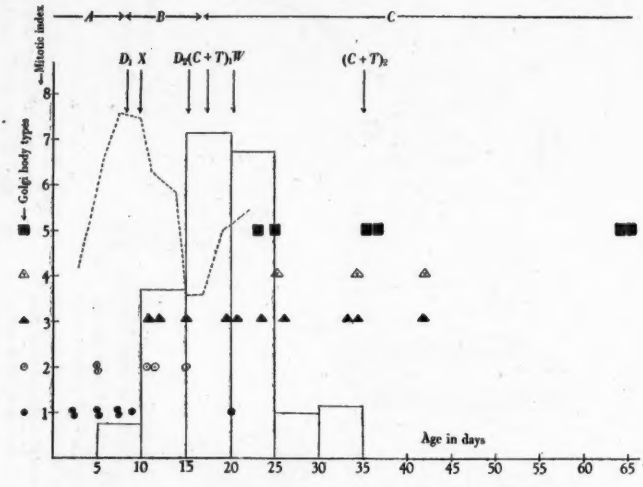

Fig. 5. The histogram is compiled from the data of Table 1. ----- Body-weight increment curve of young mice (from Gates, 1925); ● 'compact' type of Golgi body; ▲ 'unfolded' type; ■ 'varied' type; ⊙ 'transitional' type between 'compact' and 'unfolded' types; △ transitional type between 'unfolded' and 'varied' types. For D_1, D_2, $(C+T)_1$, $(C+T)_2$, *X* and *W*, see text.

probably of calcium) in the lactating mammary gland of the mouse between the 9th and 11th days of lactation (see *X*, Fig. 5)—a period which corresponds with the initial sudden increase in mitotic activity of the parathyroid. Also, although it is not known whether the percentage salt content of mouse milk decreases in the same manner as has been reported in the rat (Cox & Mueller, 1937), it has been shown (Snell, 1941) that the milk production in the mouse begins to decrease from about the 10th day of lactation. Thus a decrease in the amount of calcium ingested by the young animal during the period under consideration seemed likely. An examination of the growth increment curve of the young mouse (shown in Fig. 5), shows that the to appear in increasing proportion (D_1 and D_2 in Fig. 5). The beginning of the last stage in the cytological differentiation ($(C+T)_1$ in Fig. 5) began just at the peak period of mitotic activity and close to the time of weaning (*W* in Fig. 5), and was characterized by the presence of a few cells of the transitional and clear types. This last phase (*C* in Fig. 5) passed gradually into the adult condition and the point $(C+T)_2$ in Fig. 5 marks the approximate time at which the general cytological composition of the juvenile and adult glands was practically identical. It is thought that weaning may possibly have been concerned both in the initiation of this final phase of development and in the concomitant decrease in mitotic activity.

The cytological development of the gland and the relation between the cell types of the adult and secretory activity

The cytological development of the gland revealed two facts of importance: first, that the cell types appeared in a definite order, and secondly, that the stem and dark cells underwent division. The order of appearance of the cells: dark cells, and then clear and transitional cells together, showed that the earliest formed dark cells were almost certainly derivatives of the initial stem cells, being the products of their division and, in the case of those dark cells having a size and form similar to the stem cells, products of a direct transformation. In the more mature glands there was evidence that further dark cells might have arisen from the division of previously existing ones, since these cells were observed in mitosis and were frequently seen in small groups.

That the transitional cell was a derivative of the dark cell and in turn gave rise to the clear cell seemed probable for two reasons. First, a serial arrangement of transitional cells could be made, showing a gradual increase in size associated with a decrease in depth of staining and accompanied by a progressive flocculation of the cytoplasm. Secondly, in those glands where the Golgi elements were of the 'varied' adult type, there were the three subdivisions already enumerated (the types (1), (2) and (3) described in a previous section) as being characteristic of this, the adult type, of Golgi element; these types were associated with dark, transitional and clear cells respectively (see Fig. 4).

The changes in the Golgi bodies observed during the secretory activity of endocrine cells have been referred to elsewhere (Foster, 1942), and all that need be stated here is the generally accepted observation that during the phase of active secretion in a cell (covering the time from the formation of a secretory product until its release from the cell) there is an expansion, dispersal and commonly a fragmentation of the Golgi elements, which reaches a maximum at the time when the secretion is being released. Since such a progressive expansion of the Golgi elements, culminating in fragmentation, could be traced in the cells of the adult gland, and since these changes were associated with the passage from dark cells, through intermediate transitional forms to clear cells, it is suggested that these observations constitute evidence for a secretory cycle—the secretion product being elaborated and then released during the passage from dark cell to clear cell. This cycle is shown in Fig. 4.

Since there was no evidence for a granular secretion product in the rat (de Robertis, 1940, 1942), the monkey (Baker, 1942) or the mouse (Foster, 1943*a*), it was thought possible that a diffuse secretion might be responsible for the 'darkness' of the cytoplasm of the dark cells, and that in the case of the mouse the conversion of stem cells into dark cells was due to the accumulation of such a substance. If there was any truth in such a notion, then it might have been expected that the synthesis of such a material would have been associated with changes in the configuration of the Golgi elements of the cells concerned. An examination of Fig. 1 shows that during the period from when the dark cells first appeared (*c.* 8 days) until they became established in quantity (*c.* 16 days), the Golgi elements certainly underwent a morphological change, passing in fact from the 'compact' to the 'unfolded' type. The change, however, was widespread and was not restricted to a small proportion of the cell population such as might have become the dark cells of the mature gland, and moreover, occasional dark cells were observed in glands from young animals where the Golgi elements were still in the compact form. These two facts, therefore, did not support the supposition that these particular alterations in the configuration of the Golgi elements (associated with the development of the 'unfolded' from the 'compact' type) were concerned with the formation of a secretion, whose presence in the cells conferred the property of 'darkness' upon their cytoplasm. The changes were rather regarded as a normal phase in the morphological progress of the Golgi elements towards the 'varied' type characteristic of the mature gland.

It is considered, therefore, that the 'darkness' of the dark cells was due to the presence of a stainable, basiphil secretion *precursor*, rather than to a true secretion itself. It is further suggested that the precursor is subsequently transformed into a non-stainable, non-particulate secretion proper, the transformation being associated with (1) the transition from dark, through transitional to clear cells, and (2) the corresponding transition from the thread-like, juxtanuclear form of the 'varied' type of Golgi element to its dispersed and fragmented form. As was pointed out above, a similar sequence of changes in the morphology of the Golgi elements can in many other types of gland cell be directly associated with a secretory cycle. Thus, on the basis of the interpretation given above, the dark cells with their juxtanuclear Golgi elements were looked upon as representing a presecretory phase; the transitional cells with their clearer cytoplasm and expanding Golgi bodies and the clear cells with their empty looking cytoplasm and broken up Golgi material were, respectively, regarded as phases of secretion elaboration and secretion release.

SUMMARY

1. Up to the age of 8 or 9 days from birth, the parathyroid gland of the mouse presents a uniform parenchyma of stem cells, with dark cells occurring occasionally in the older glands. The Golgi bodies are juxtanuclear in position and for the most part compact in form, consisting of osmiophil threads or networks.

2. From about 9 days onwards, there is an increasing vascularity, accompanied by the appearance of dark cells in increasing proportions. At the same time the Golgi bodies, still juxtanuclear in position, begin to 'unfold' and present a more elaborate form. These changes are widespread throughout the glands and the 'unfolded' type of Golgi element is regarded as a well-defined transitional stage between the 'compact' and 'varied' types.

3. The above changes run parallel with an increase in the mitotic activity of the gland: an activity which reaches its climax by the end of the 3rd week after birth. A calcium deficiency in the maternal milk is suggested as a likely stimulus causing the rather sharp increase in the mitotic index at this stage in development.

The bulk of the cells undergoing division are stem cells, but there is also evidence for the division of dark cells on a more limited scale.

As the peak of mitotic activity is reached, the gland begins to assume an adult appearance, with the production of transitional and clear cells. At this stage the Golgi bodies begin to present the diversity of form characteristic of the adult 'varied' type.

4. The 'darkness' of the cytoplasm of the dark cells is thought to be due to a secretion precursor rather than to the presence of a secretion proper.

5. The following secretory cycle is suggested for the mature gland: *dark cells* (containing a secretion precursor) → *transitional cells* (in which elaboration of true secretion occurs, presumably in whole or in part from the precursor) → *clear cells* (release of secretion into the circulation).

Note. A recent paper by Baker (1945) dealing with cytological changes in the rat parathyroid has come to the writer's notice, in which the author suggests that the dark cells of that gland represent cells in a condition of secretory exhaustion. If, as seems likely, these are homologous with similar cells in the mouse, such an hypothesis seems improbable since, as has been pointed out, these cells (in the mouse at all events) appear quite early in post-natal development when the gland shows little cytological sign of activity.

The writer would like to thank Dr J. H. Woodger for his interest in this work and Messrs P. C. Williams and A. Hepple of the Courtauld Institute of Biochemistry, Middlesex Hospital, for providing some of the animal material.

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RADIAL HEMIMELIA AND THE FUNCTIONAL ANATOMY OF THE CARPUS

By R. O'RAHILLY. *Department of Anatomy, University College, Cork*

The author's attention was directed recently to the hands of a woman aged about 50 years. Clinical and radiological examinations were made and an unusual combination of malformations thereby discovered.

The patient is employed as a cook and carries out her duties without difficulty. She can hold a pencil between the index and middle fingers of her left hand and can sew as well as write. Her feet are normal. She knows of no relative with any similar condition.

DESCRIPTION OF CASE

Left hand. The first digit of the left hand resembles a finger rather than a thumb in appearance; it has three slender phalanges, which, however, are rotated medially in relation to the plane of the palm as are those of a normal pollex. The web between the thumb and the index finger is diminished and the first metacarpal bone cannot move or be moved independently of the second. The first phalanx of the thumb has a normal range of movement. The second phalanx can be flexed to a right angle and extended through about 35°; these movements can be performed actively or passively. The third phalanx of the thumb cannot be moved actively; passively it can be flexed through about 20° from the extended position. The whole thumb can be *apposed* actively to the distal phalanges of the four fingers by flexion, but there is no true *opposition* without forcibly rotating the pollex. There is no thenar eminence. The left wrist is narrower than the right (due chiefly to the radius and the soft tissues surrounding that bone), but the wrist movements are normal on both sides.

Right hand. The first digit of the right hand is underdeveloped and is connected to the hand merely by soft tissues. It lies across the base of the index finger when at rest but it can be made to move about passively; it has no active movement. It consists of three bony segments and has a proximal joint which appears ankylosed clinically and a distal joint which can be flexed passively through a right angle.

RADIOLOGICAL EXAMINATION

A dorso-palmar skiagram (Pl. 1, figs. 1, 2) shows the following features:

Left hand. The radial styloid process has not developed.

The trapezium and the distal part of the scaphoid are absent. The capitate and trapezoid are fused, and the latter bone appears to articulate with both the first and second metacarpals. The pisiform is unduly proximal.

The first metacarpal is long and slender, resembling that of the other fingers. It carries three slender phalanges distally and appears to articulate proximally with the trapezoid.

This first digit might be considered as a triphalangeal thumb which became attached to the trapezoid in the absence of the trapezium, or it might be looked upon as a duplication of the index finger with absent thumb. A review of the literature of anomalous thumbs has induced the writer to agree with Windle (1891) in regarding the distinction between a finger and a thumb in such cases to be more notional than real.

Right hand. The radial styloid process has not developed, as in the left hand, and the lower end of the radius is indented by the small scaphoid and shows a localized rarefaction proximal to this depression.

As in the left hand, the trapezium and distal part of the scaphoid are absent. The capitate and trapezoid are also fused and the pisiform is unduly proximal.

Three pieces of bone are seen on the radial side, not connected to the skeleton; these have been rotated laterally for the skiagram but, as mentioned already, they lie across the palm usually. Two slender phalanges are present and these articulate with a spicule of bone proximally; this spicule may be regarded as part of a proximal phalanx of a triphalangeal digit (as in the left hand) or as part of a first metacarpal of a biphalangeal digit (as in a normal hand).

In both hands the index fingers have a compensatory ulnar deviation (clinodactylia) to aid the grip between the index and middle fingers.

OTHER CASES

Bergerhoff (1927) illustrates a case in which the radius, scaphoid, and trapezium are absent. The capitate and trapezoid are fused and the first two metacarpals articulate with the trapezoid. The first digit is biphalangeal.

Cases showing a 'floating thumb' are rare. The commonest floating digit is a pedunculated post-

minimus; these digits have been described by Cummins (1932). De Pereira & de Lima (1930) show a nodule representing a supernumerary fifth metacarpal with two phalanges, the appearance of the extra digit closely resembling the right thumb of the present case. Of floating thumbs, however, the author has found only four cases reported in the literature which are described in detail sufficient to serve for comparison with the present case.

Klippel & Rabaud (1903) illustrate a left hand with a floating thumb consisting of two phalanges and a spicule (Text-fig. 1*b*). The trapezium is reduced and the trapezoid and capitate are not fused. The distal end of the scaphoid is absent, but this bone is distinctly larger than in the present case. The radiostyloid has not developed. The right hand is normal except that the radiostyloid did not develop.

Botreau-Roussel (1922) describes a left hand in which the thumb is floating. The scaphoid is absent and the trapezium reduced, and there is no radiostyloid. The right thumb is normal but the scaphoid is absent.

Bergerhoff (1927) shows a right hand with a floating thumb, in which the radius, scaphoid, and trapezium are absent.

Fontes (1930) describes a right hand having a floating thumb in which the radius and scaphoid are absent. The trapezium and trapezoid are reduced. The left hand has neither thumb nor radius, and the carpus consists of fused masses.

RADIAL HEMIMELIA

It will have been noticed that these deformities of the thumb are frequently associated with defects of the radius. Radial hemimelia, according to Bunnell (1944), is a defect 'of the radial bud and, therefore, often includes the carpal and digital rays of the radius and the radial part of the end of the humerus'. The thumb is usually absent and in some cases the index finger also is affected; in rare cases the ulna is double. The soft tissues are very frequently involved in these deformities. The condition is relatively common; Kato (1924) collected 253 cases from the literature up to 1923 and since then many more have been added. In adult cases the condition of the carpus can be determined from a skiagram; the vast majority of cases, however, have been described in infants under 1 year of age and nothing is known about the carpus except in the few instances where a dissection could be made. The writer has investigated 41 limbs from the literature, in which the radius was absent (at least distally) and in which the carpus was either illustrated or described as a result of investigation by radiography or dissection.

Two of these limbs had only one finger each together with one and no carpal bone respectively. Of the remaining 39 cases the scaphoid was absent in 32 and reduced in one; the trapezium absent in 31 and reduced in two; the first metacarpal absent in 31 and reduced in three; the thumb phalanges absent in 29 and reduced in five. Each of these bones named may be present, therefore, in some cases of absent radius; all were simultaneously *absent* in 26 of the 39 cases (67·0%). These bones appear to be usually, but not necessarily, dependent on the radius.

By contrast, a consideration of the neighbouring carpal bones in cases of absent radius showed that the lunate was absent in only three out of 34 cases and the trapezoid in four out of 35; both were simultaneously *present* in 29 out of 34 cases (85·3%).

A further series of cases from the literature was considered, in which the distal part of the radius was present but in which an abnormality of the scaphoid, trapezium, or thumb occurred. In thirteen such cases (pure phalangeal defects were excluded) the radiostyloid was absent in six, definitely present in two, and not recorded in five; the scaphoid was absent in seven and reduced in five; the trapezium absent in five and reduced in four; the first metacarpal absent in four and reduced in five; and in four of the cases there were no thumb phalanges. In all cases where the scaphoid was present, either the trapezium or the first metacarpal and its phalanges were present also. These cases (among which the present case is included) are regarded as less extensive varieties of radial hemimelia, in which the radius is affected only in its styloid process, if at all.

FUNCTIONAL SUBDIVISION OF SCAPHOID

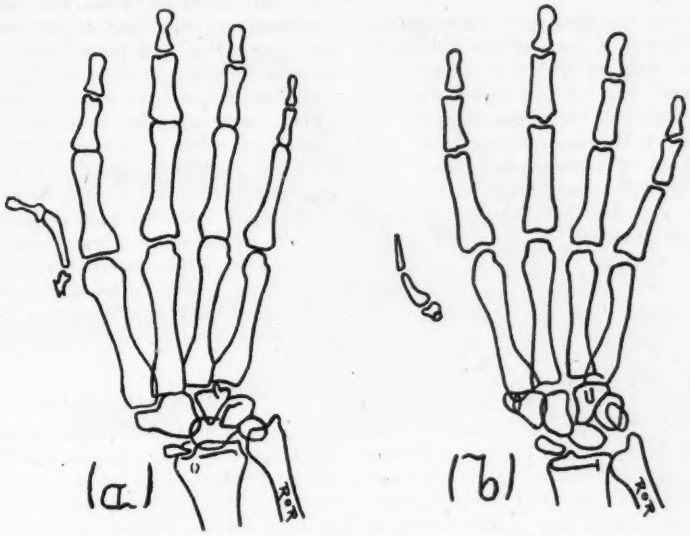
A functional subdivision of the carpus has been described by MacConaill (1941) in which the scaphoid is separated from the other bones of the proximal carpal row: the division is 'based upon the observed fact that this bone moves at times with the proximal and at times with the distal row'. The scaphoid first moves *with* and then *upon* the proximal row during progress from palmar flexion to dorsiflexion. It is implied that the scaphoid may be divided functionally into three parts:

(1) A part which articulates with the lunate.

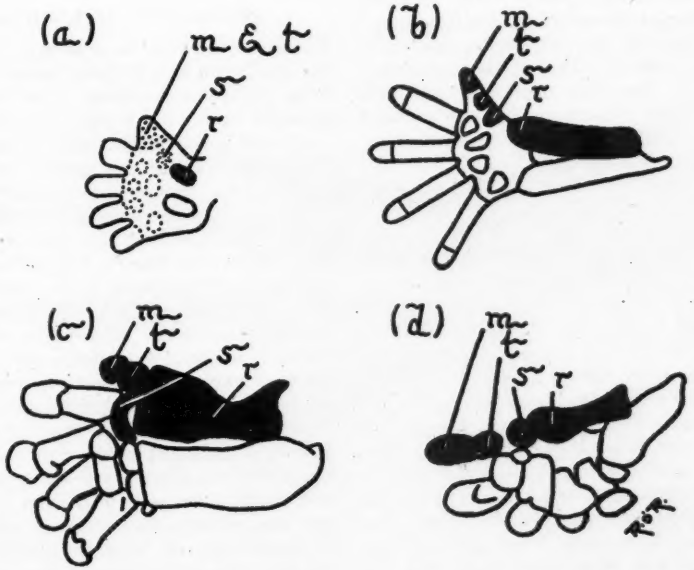
(2) A part which articulates with the capitate and trapezoid.

(3) A part which articulates with the trapezium.

The functional anatomy of the first two parts has been dealt with by MacConaill (1941). The second and third parts seem to occupy the position of the centrale. The third part of the scaphoid, together with the trapezium, appears to form part of an osseo-articular system which is functionally and



Text-fig. 1 (*a*) Author's case (right hand), and (*b*) Klippel & Rabaud's case (left hand).



Text-fig. 2. Reconstructions of embryos showing development of trapezium (after (*a*) and (*b*) Lewis, (*c*) Hagen, and (*d*) Gräfenberg). *r*, radius; *s*, scaphoid; *t*, trapezium; *m*, metacarpal 1. Radial ray black (cartilage) or stippled (pre-cartilage).

ontogenetically separable from the remaining carpalia and digits.

In the present case the third part of the scaphoid is absent in both hands, and neither thumb has normal function. This is also the condition in Klippel & Rabaud's case (already mentioned), but there the second part of the scaphoid is present whereas it is absent in the present case (Text-fig. 1). The trapezoid is not left unsupported in the latter instance, however, but is fused with the capitate; it is to be noted that fusion of the trapezoid and capitate has been described in otherwise normal hands, e.g. by Cave (1926).

TRAPEZIUM AS FUNCTIONAL CONSTITUENT OF THUMB

Bunnell (1944) maintains, from radiological investigations on the wrist, that in lateral movements of the hand the line of the middle carpal joint runs through the cleft of the thumb instead of passing between the scaphoid and trapezium; he concludes that the thumb can be considered 'as a prolongation of the proximal carpal row'. Fick (1911), in considering the function of the thumb as the lesser hand (*die kleine Hand*) of Albinus, does not refer to the trapezium as a constituent of its mechanism. Terry (1943) has stressed the peculiar position of the trapezium in the carpus as influencing the movements of the thumb. Bunnell (1938) concludes from his work on opposition that most of the angular motion takes place at the carpo-metacarpal joint and most of the rotary motion at the metacarpo-phalangeal joint, but also mentions that the trapezium moves with the thumb on the rounded distal end of the scaphoid and that the latter bone angulates forwards during opposition.

DEVELOPMENT OF TRAPEZIUM

Embryological considerations appear to indicate that the trapezium is developed as a constituent of the thumb. The development of the upper limb has been described by Lewis (1902). The arm bud forms about the third week and begins to differentiate during the fourth week, the skeletal core appearing as cartilage by the fifth week. The carpus and fingers are first seen as centres of increased condensation in the general mass of mesenchyme. Lewis illustrates a model of an embryo at this stage (Text-fig. 2*a*; 10·5 mm.), in which the carpal bones have appeared; the metacarpals and phalanges have not yet differentiated from each other, and the presumptive site of the trapezium (which has not differentiated) is occupied by that mass of mesenchyme which forms the thumb. Lewis then illustrates a later stage (Text-fig. 2*b*; 16 mm.) in which several of the carpal bones have become chondrified and in which the proximal phalanges of the fingers are differentiating from the metacarpals; the cartilaginous trapezium is now distinct from the first metacarpal but both pieces of cartilage still occupy a position in the same digital ray.

A comparison with other reconstructions, however, 'shows that there is considerable variation in the carpal region'. Hagen (1900) represents the first metacarpal as coming in contact with the radius, but it would seem more probable, as both Lewis and Gräfenberg have pointed out, that what he calls metacarpal 1 is trapezium and his so-called first phalanx the metacarpal (Text-fig. 2*c*; 17 mm.). Gräfenberg (1905) shows an embryo (Text-fig. 2*d*) confirming the close association of the developing trapezium with the first metacarpal.

LIMB RAYS

From the functional point of view the radius, scaphoid, trapezium, first metacarpal, and thumb phalanges form a radial ray, each constituent of which appears to be necessary for the fully functioning human pollex. Longitudinal limb rays appear to have been mentioned first by Gegenbaur in 1876 in connexion with his morphological theories; Pfitzner thought that the longitudinal arrangement was interrupted at the wrist.

AETIOLOGY OF LIMB DEFECTS

Experimental work on limb rays has been carried out on Triton and Proteus larvae and on Amphioxus. According to Huxley & de Beer (1934): 'the preaxial border of the limb (marked by the first digit and radius in the fully developed limb) will arise from the anterior portion of the limb-disc. Although the limb-field is regionally still undetermined, it is polarised along the anteroposterior axis from the first moment at which its existence can be detected: this is proved by grafting limb-discs in abnormal orientations.' This conclusion is significant in considering the production of various types of radial hemimelia. Such defects are usually attributed to a site of focal deficiency, that is, an area where a congenital or hereditary malconstitution of the germ-plasm results in imperfect histogenesis. When this aberration of development affects the polarized limb-field before regional differentiation has occurred a greater or lesser defect of a limb ray may result. The parts of the ray above and below the affected area may develop normally in some instances and an intercalary form of defect be produced, as in the present case; a less extensive example of the intercalary type is described by Hodgson (1943). Various other factors involved in the aetiology of limb defects are discussed by Kato and Bunnell.

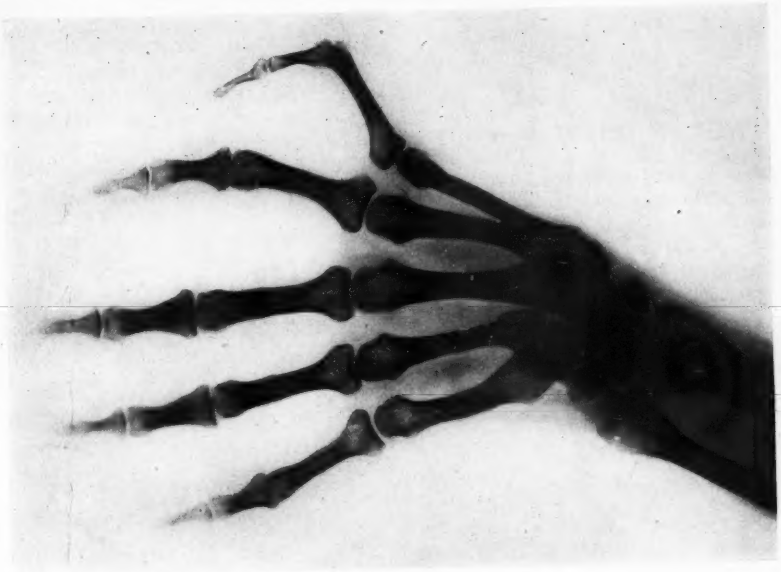


Fig. 1

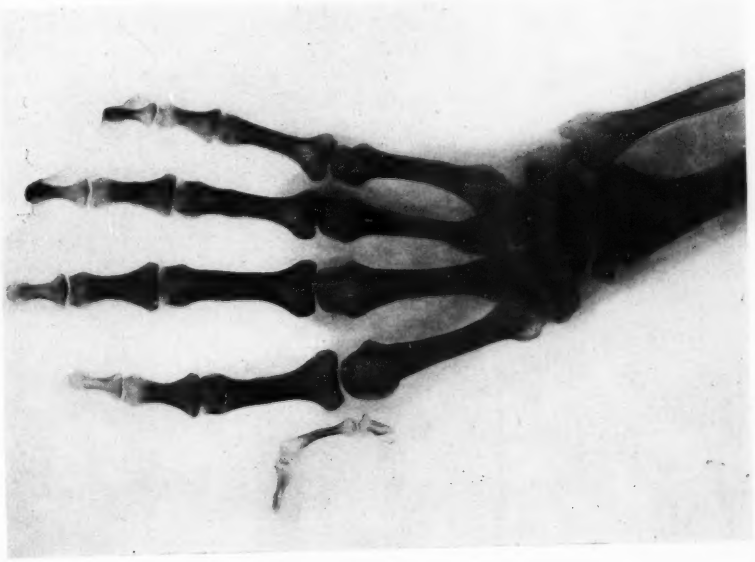


Fig. 2

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SUMMARY AND CONCLUSIONS

1. A description is given of a case of 'floating thumb' associated with other congenital anomalies of the hands. Clinical and radiological examinations were made. No hereditary history was elicited.

2. Relevant cases in the literature are cited. A study of radial hemimelia shows that in cases of absent radius the scaphoid, trapezium, first metacarpal, and thumb phalanges are usually absent but that all other bones of the hand are usually present. The present case is regarded as an intercalary form of radial hemimelia.

3. A comparison of this case with that of Klippel & Rabaud (1903) emphasizes the functional subdivision of the scaphoid into three parts, suggested by MacConaill (1941).

4. The peculiar position of the trapezium in the carpus and the role of this bone in movements of the normal thumb indicate that the trapezium is a functional constituent of the human pollex.

5. Embryological reconstructions seem to show that the trapezium is developed as part of the thumb.

6. The radius, scaphoid, trapezium, first metacarpal, and thumb phalanges form a functional limb ray. The normal functioning of the human pollex may be interfered with by maldevelopment of any constituent or constituents of this ray. These defects occur probably as areas of imperfectly constituted germ-plasm affecting the polarized limb-field when it is still regionally undifferentiated.

The author is indebted to Miss K. Abbott, D.M.R., for taking the skiagram, and to Prof. M. A. MacConaill for his helpful advice and criticism.

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THE PLANE OF FRACTURE OF THE CAUDAL VERTEBRAE OF CERTAIN LACERTILIANS

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Certain lizards have the power of producing self-mutilation by severance of the tail; this autotomy is followed in the course of time by regeneration. The phenomenon is a well-known one, but a thorough scientific examination of the actual anatomical conditions that make it possible has not been carried out. Its occurrence in *Sphenodon* was first described by Günther (1867), and later by Howes & Swinnerton (1901). Its general presence among the Lacertae and Geckones has attracted the attention of investigators such as Leydig, Gegenbaur, Hyrtl, Müller and many others. In spite of the natural interest that this phenomenon has excited, the literature on the subject is full of misleading and confusing statements, and it is with the object of clearing up this confusion that the present investigation has been undertaken.

It is generally agreed that this highly specialized protective mechanism on the part of the lizard is a result of adaptation to prevailing environmental conditions (Morgan, 1901). It is of interest that it does not occur in any species in which the tail possesses a definite and specialized function, such as swimming or grasping, since under these conditions it would prove to be a distinct disadvantage by causing mechanical instability. Associated with the development of the power of autotomy there is a corresponding increase in length of the tail. Boulenger (1920) states that the more primitive members of a genus possess a relatively shorter tail in relation to the length of the body than do the more specialized species.

In order that autotomy may occur, a predetermined fracture plane is developed across the tail. Usually this takes the form of a series of such planes, resulting in a segmented condition. The actual relation of such planes to the vertebra has caused a certain amount of confusion. Most investigators, such as Gadow (1901, 1933), Cope (1892, 1900) and Woodland (1920), state that the plane passes through the centrum of the vertebra, dividing it in some cases into equal and in others into unequal portions. Leighton (1903) claims that in *Lacerta viridis* and *Anguis fragilis*, at least, there is an intervertebral fracture plane. His evidence for this statement, however, is not very convincing.

Those morphologists who believe in the intracentral fracture plane are further divided in their views as to the exact relationship of the fracture plane to the constitution of the vertebra itself. A fracture plane passing approximately through the middle of a vertebral body would suggest an intersegmental condition in the true morphological sense. Goodrich (1930) accepts this, by explaining that it is due to the failure of fusion of the caudal and cephalic halves of adjacent sclerotomes. Gadow (1933), with his views on vertebral constitution, considers it not to be truly intersegmental, since if it were, it would occur between the basiventral and interventral elements, that is, between the centrum and the intervertebral disc. This would support Leighton's intervertebral fracture-plane theory.

The origin of the vertebral fracture plane, as given by Gadow (1933), is that a cartilaginous septum develops in the middle of the vertebra, destroying the notochord. This is often known as the 'chordal cartilage'. Gegenbaur (1862) and Hyrtl have both shown that the split which is often present in the vertebra develops only after ossification has taken place. It commences on the outside, becomes deeper, and then extends to the neuro-central suture and even to the neural arch. This split, though rendering autotomy more simple, is not essential, since, according to Cope (1892, 1900), it is not present in *Ophisaurus* (the glass snake), which shows the phenomenon in a high state of perfection.

The septum, which appears to represent an intersegmental structure, is almost certainly secondary in nature. Goodrich (1930) points out that it is not seen in any of the primitive reptiles, while Gadow (1933) and Willeston (1925) state that it is a post-embryonic or at least a late embryonic development.

Having considered the general morphological interpretation of the fracture plane, the structural details associated with the occurrence of autotomy will be described. The present investigation has been confined to two indigenous species, *Lacerta vivipara* and *Anguis fragilis*. A careful description has been made by Woodland (1920) of the Indian Gecko (*Hemidactylus flaviviridis* Rüp.), which makes a useful comparison.

The most anterior caudal vertebrae possess no split in their centra and form the basal unsegmented region of the tail. Such a condition is essential for the protection of the cloaca. The first vertebra to show the presence of a split is either the seventh or the eighth and, in *Sphenodon* at least, it is not

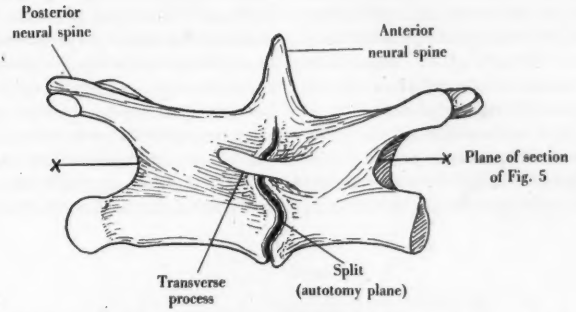

Fig. 1. *Lacerta vivipara.* Mid-caudal vertebra, lateral aspect. ×22·5.

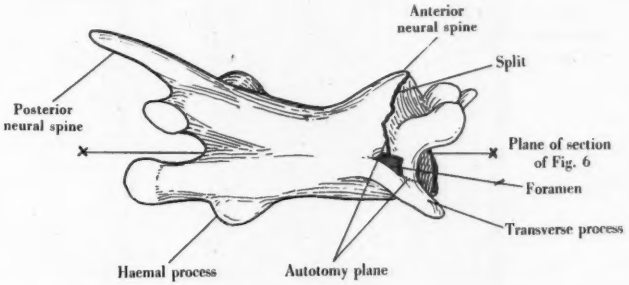

Fig. 2. *Anguis fragilis.* Mid-caudal vertebra, lateral aspect. ×10.

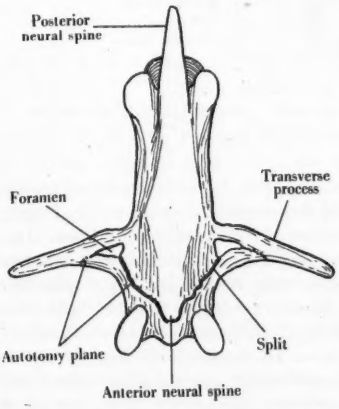

Fig. 3. *Anguis fragilis.* Mid-caudal vertebra, dorsal aspect. ×10.

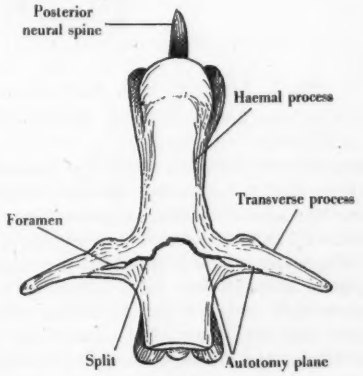

Fig. 4. *Anguis fragilis.* Mid-caudal vertebra, ventral aspect. ×10.

constant for a species, occurring from the sixth to the eighth vertebra (Howes & Swinnerton 1901).

In *Lacerta vivipara*, the split divides the centrum and extends into the neural arch almost to the neural spine (Fig. 1). Here it ceases and if autotomy occurs, it causes direct fracture of the bone. The split divides the vertebra approximately into halves in the mid-caudal region, though cephalad it may occur between the anterior and middle thirds of the vertebra, but it always passes posterior to the transverse process. The split is bordered by well-marked lips which may be due to the growth of the vertebra after formation of the split. Howes & Swinnerton (1901) claim that in *Sphenodon*, on the ventral surface, these lips provide additional protection to the haemal canal. The lips are bound together by means of the dura mater which issues from the vertebral canal, passes through the split, blends with the periosteum and, outside the vertebra, becomes continuous with the tissue of the autotomy plane (Fig. 5). Thus when autotomy occurs, this tissue is torn away from the bone.

In *Anguis fragilis* (Figs. 2–4) the conditions are somewhat different. The split extends across the centrum and across the neural arch, including the neural spine (the 'anterior neural spine' of Cope, 1900). Laterally, it reaches the anterior border of the foramen which partly divides the transverse process at its base. Thus when autotomy occurs, the only part actually fractured is the anterior part of the divided base of the transverse process, resulting in a short transverse process retained by the animal and a long process attached to the lost tail. Unlike the condition present in *Lacerta*, the split occurs very near the anterior end of the vertebra; approximately a quarter of the vertebra is divided from the rest in autotomy, and this occurs both in the anterior and mid-caudal regions. The split is not quite as wide as in *Lacerta* either in the centrum or in the neural arch or spine, but is more like a crack in the bone. Its constant presence and the fact that the periosteal covering is intact show that it is not solely a result of mechanical strain, though probably it is brought about in this way early in life and long before autotomy occurs. There is no continuance of tissue from within the vertebra as in the case of *L. vivipara*, even in the more marked region of the split, though if the split is not very wide there is a continuance of periosteum across it (Fig. 6).

It is interesting to note that in both species the

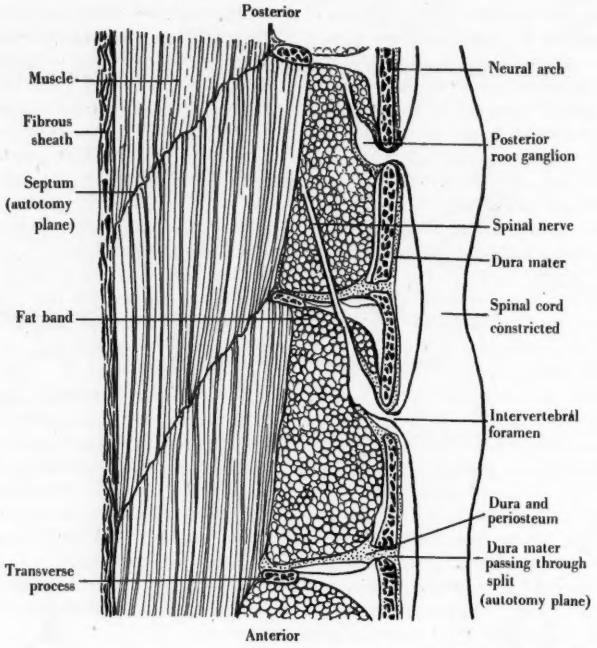

Fig. 5. *Lacerta vivipara*. Horizontal section of mid-dorsal region, through plane of transverse process (only one side shown). ×22·5.

spinal cord itself shows marked constriction at the fracture plane (Figs. 5, 6), a possible means of ensuring minimal damage. The relation of the spinal nerve is essentially the same in both species. On leaving the intervertebral foramen, the anterior and posterior roots join to form the spinal nerve, which passes backwards above the transverse process and across the fracture plane where, at least in the case of *L. vivipara*, its sheath of dura mater is continuous with the septal tissue (Fig. 5). The nerve passes within the 'fat band' to the next segment which it supplies. Woodland (1920) states that the regenerated tail may be supplied by the last two or three spinal nerves, which suggests that one nerve may supply several segments.

The importance of the special arrangement of the muscles is emphasized by Woodland (1920) and others, for without this, autotomy could not occur. This is really the most important part of the mechanism of autotomy, for if the bone is fragile, it will readily fracture without any preformed split and this undoubtedly often occurs.

In each species the arrangement is similar. Due to the presence of fibrous septa, the muscles are arranged into eight bands, two ventrally, four laterally and two dorsally, and these all interdigitate with those of the next segment. The posterior attachment is to the vertebra and to the septa covering the fat bands, while the anterior attachment is largely into the next segment via the septal tissue. Thus when autotomy occurs, the proximal end of the lost tail has eight pointed muscle bands protruding.

In *L. vivipara*, the anterior attachments of the lateral muscle bands are made to the transverse process by means of a mass of septal tissue (Fig. 5), while in *A. fragilis* there is a direct attachment to the transverse process of the next segment (Fig. 6) which results in the division of the process on fracture.

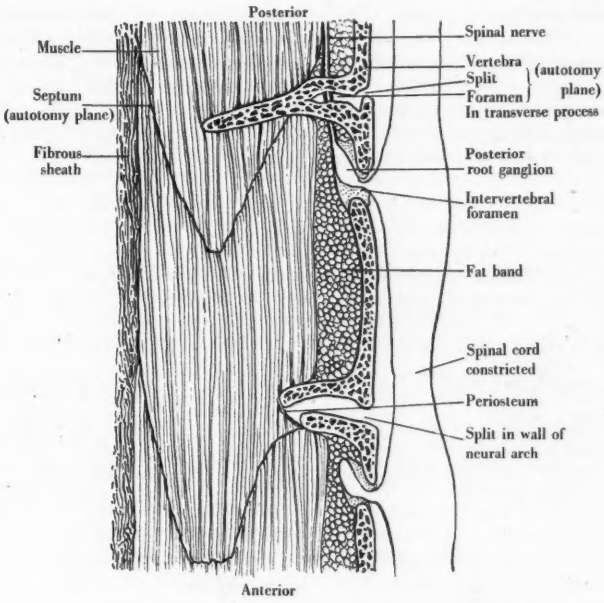

Fig. 6. *Anguis fragilis*. Horizontal section of mid-dorsal region, through plane of transverse process (only one side shown). ×10.

The apparently unstable attachment of the anterior ends of the muscle bands into septal tissue can be understood when it is realized that, in ordinary movement of the tail, the tension produced by the muscular contractions is equally distributed throughout all the segments. However, when one part of the tail is fixed, as occurs when it is caught by a bird, the undue strain produced by the movement of the rest of the tail in front of the fixed part results in fracture.

The relation of the fracture plane to the scales is well known, occurring in most cases between every two rows of scales, a fact that can be made use of in determining possible planes of fracture, as in the basal region where scaling is irregular, no fracture

plane exists. Beneath the scales is a subcutaneous fibrous sheet which is continuous with the septal tissue and shows no indication of segmentation. It is somewhat thicker and far stronger in *A. fragilis* (Fig. 6) than in *L. vivipara* (Fig. 5).

The integrity of the tail can now be considered, for though at times it may be a great advantage to undergo autotomy, it must be possible to sustain certain mechanical strains without fracturing. In *L. vivipara* a protection against excessive autotomy is present in the integument, but much more important is the fibrous tissue binding the lips of the vertebral split together and the dura mater which blends with the periosteum. The strong subcutaneous fibrous sheet seen in *A. fragilis* affords great protection to the tail, in fact, it is very difficult to break the tail by simple mechanical strain, while in *L. vivipara* the tail appears by comparison very fragile. It is interesting to note that there is not the strong fibrous sheet binding the split as there is in *L. vivipara*, for any strain the well-protected vertebra might undergo would be met by the thin periosteal covering and the undivided transverse process.

In conclusion, it is found that certain lizards have developed a special caudal fracture plane and that morphologically this plane is almost certainly intersegmental. Those species which show the development of this plane exhibit the phenomenon of autotomy. The nature of the plane is not as stated by Woodland (1920), a hyaline septum, making a continuous cleavage plane, save for blood vessels, nerves and spinal cord; but rather is a partial cleavage (or fracture) plane of most of the tissues of the tail which is compensated for by the strengthening of other tissues in order to prevent excessive autotomy. The amount and position of the strengthening material, however, vary considerably with different species.

I am indebted to Prof. F. Wood Jones for his advice and assistance.

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SPIRAL NERVE ENDINGS IN THE EXTRINSIC EYE MUSCLES OF MAN

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The extrinsic muscles of the eye differ from other somatic muscles in morphology, in physiological response and not least in their mode of innervation. Most studies of the motor nerve endings in the ocular muscles have been made on non-human material, and methylene blue staining has been the method of choice. It is probably due to these facts that the peculiar spiral encircling terminations of large numbers of nerve fibres in the extrinsic eye muscles of man, described in this paper, have not been previously reported. A review of the literature on the structure and innervation of the extrinsic eye muscles up to 1936 is given by Irvine (1936).

The presence of proprioceptive endings in ocular muscles was first postulated by Sherrington in 1897. He expected to find these muscles, with their great delicacy of control and co-ordination, well supplied with muscle spindles of the type found in other somatic muscles (Kuhne, 1863). He was, however, unable to find such structures, though later Tozer & Sherrington (1910) mentioned simple endings as occurring in the extrinsic eye muscles of rabbit, cat and monkey, which they regarded as receptive spindles of peculiarly simple form, but which unfortunately they neither described nor illustrated.

It is now generally accepted, on morphological grounds, that there are afferent nerve fibres in the extrinsic muscles of the eye and that they have two main types of ending, both situated mainly in the anterior third of the muscles: (*a*) an ending somewhat resembling the neuro-tendinous organ of Golgi at the anterior musculo-tendinous junction (Marchi, 1882; Sherrington, 1897; Huber, 1900; Dogiel, 1906*b*; Tozer & Sherrington, 1910); and (*b*) the *terminaisons en grappe*, epilemmal grape-like endings or atypical motor endings of Retzius (Retzius, 1892; Huber, 1899; Hines, 1931; Woollard, 1931, 1937). The motor nerves, typical thick medullated fibres having characteristic motor end plates (Pl. 2, fig. 11) and hypolemmal endings, terminate mainly in the middle third of the muscle. The only other nerve fibres are the fine sympathetic fibres going to blood vessels.

Tozer & Sherrington (1910), using cats, rabbits and monkeys, cut the oculomotor nerves and found that all the intramuscular myelinated nerve fibres degenerated, and they therefore suggested that these nerves are mixed. They severed also the first division of the 5th nerve in several experiments and found very occasionally a degenerated nerve fibre in the eye muscles. They concluded that though the 5th nerve may send a few fibres to the muscles in the orbit they are insignificant in number in the animals employed. Hines (1931) in a detailed and important study of the extrinsic eye muscles of the rabbit obtained essentially similar results. Tarkhan (1934) cut the oculomotor and trochlear nerves in cats at their exit from the brain and found degeneration of all motor and sensory nerve fibres and endings in the muscles supplied, apart from a few minute fibres which, he considered, arose from cells distal to the cut and which he concluded were not numerous enough to account for proprioception. He thus agreed with Tozer & Sherrington (1910) that these nerves were mixed. His further observations suggested that fibres from these nerves ran in the tectospinal tract to the mesencephalic nucleus of the fifth nerve.

Abd-el-Malek (1938) added physiological proof that some of the fibres of the oculomotor and trochlear nerves are sensory by stimulating the central ends after severance of these nerves and finding a significant rise of blood pressure. Stimulation of the peripheral end caused no rise of pressure.

Corbin & Oliver (1942) destroyed the mesencephalic root and nucleus of the 5th nerve with a Clarke-Horsley apparatus and found that there were no degenerated myelinated fibres in the 3rd, 4th or 6th nerves. They also destroyed the nucleus of the 4th nerve and found that all nerve endings in the superior oblique degenerated. They therefore concluded that the cells of origin of the grape-like endings were probably intermingled with the motor cells of the oculomotor nerves, and did not end in the mesencephalic nucleus of the 5th nerve as Tarkhan's (1934) observations suggested. Thus the evidence presented in these papers indicates that nerve fibres to and from all types of nerve endings in the extrinsic muscles of the eye run together in the oculomotor nerves and probably originate or end in the nuclei of these nerves.

Reference may now be made to a few other papers reporting various types of muscle spindles. Cilimbaris (1910), working on the sheep, rabbit and cat, described and figured typical muscle spindles with two to twenty muscle fibres in the spindle, and Pallot (1934) found in the cat simple neuro-muscular spindles of the Kuhne type, which he illustrated

rather unconvincingly. Sutton (1915) found in the eye muscles of pig embryos a neuro-fibrillar net with plaque-like endings, and in addition a neuro-muscular spindle. Hines (1931) was not convinced by the descriptions and drawings in this paper, and it is impossible on studying the paper not to share this lack of conviction. Dogiel (1906*a*) described complex networks of fine nerve fibres around muscle fibres, but again the methylene blue pictures do not make the actual morphology clear. Hirano (1940) figured encircling nerve fibres of rather complex type around some muscle fibres, but on purely morphological grounds he considered these to be motor endings. He also regarded the *terminaisons en grappe* as being motor endings, thus differing from most observers. Fulton (1943) and Weddell, Harpman, Lambley & Young (1940) express the present view that there are no neuro-muscular spindles in the extrinsic eye muscles. It is clear that these muscles have an innervation unique both in peripheral arrangement and in central origin, though there is still not absolute agreement amongst workers with regard either to morphology or function, and it is evident that certain of the spiral encircling nerve endings illustrated in this paper have not been observed previously in these muscles either in man or other animals. A study of the comparative anatomy of the nerve endings is now in progress.

MATERIAL AND METHODS

The extrinsic eye muscles were removed during post-mortem examination, within 24 hr. of death, from subjects in which there was no evidence or history of disease of the nervous or muscular system. The roof of the orbit was removed, the anterior clinoid process and the bone around the optic foramen being left intact. These, with the attached origins of the muscles to the annulus tendineus, were then removed in one piece. The globe of the eye was then cut through anterior to the equator, to secure the insertions of the muscles while leaving the anterior part of the globe *in situ*. The posterior part of the globe with muscle cone and bony origin was then removed and fixed *in toto* in 10% formol saline. After fixation the muscles were dissected out, orientation being secured by means of the intact anterior clinoid process. Some muscles were embedded in celloidin, some in paraffin and others reserved for frozen sections. Longitudinal and transverse sections were made, the sections being from 7 to 60 μ in thickness.

Sections were stained with haematoxylin and eosin, haematoxylin and van Gieson, gallocyanin, Mallory's phosphotungstic acid haematoxylin, Masson's trichrome method, Weigert-Pal, del Rio Hortega's double impregnation method for reticulin fibres, and by two modifications of the Gros-Bielschowsky method, that of Weddell & Glees (1941) and that of Denny-Brown (1938). In addition Holmes's (1942) method and a modification kindly suggested by Dr Holmes were used. Whilst using the Gros-Bielschowsky method a number of sections were left by mistake in sodium thiosulphate ('hypo') for 48 hr. after being toned in gold chloride. It was found that the faint background staining, which is usually present in this method, had been completely abolished, the nerve fibres standing out sharply on unstained background, and this modification was subsequently used with great success.

OBSERVATIONS

The oculomotor nerves enter the muscles in the region of the junction of the middle and posterior thirds, and quickly break up into nerve bundles of decreasing size which pass across and along the muscle fibres. The number of these nerve bundles is so great that a muscle stained by a Gros-Bielschowsky method shows to the naked eye a speckled black band, up to about 0·5 cm. wide, stretching across the mid-zone of the muscle. Great numbers of thick medullated fibres from these small nerve bundles spread out fan-wise to end in the middle third of the muscle at typical motor end-plates (Pl. 2, fig. 11). But in this region also many medullated nerve fibres have the following arrangements at their termination:

(*a*) *Simple spirals.* The nerve fibre, surrounded by its Schwann and myelin sheaths, takes from three to eight complete spiral turns around a single small muscle fibre to end in fine finger-like processes in an end-plate, resembling a motor end-plate (Pl. 2, figs. 8–10), or in some cases seeming to end in a single fine point with no relation to a nucleus (Pl. 1, figs. 1, 2, 3 and 6).

Thick transverse sections stained by the Gros-Bielschowsky method show these encircling fibres as single black spiral rings (Pl. 1, fig. 5) quite different from the appearance of the reticulin network around the fibres (Pl. 4, fig. 19). Weigert-Pal sections show a picture (Pl. 1, fig. 7) similar to Gros-Bielschowsky preparations. Thick sections treated with any of the nuclear stains show a number of muscle fibres each encircled by a spiral ring of Schwann nuclei, and it becomes possible with experience to distinguish muscles encircled by nerve fibres as easily in haematoxylin and van Gieson preparations as in those stained by specific axon-staining methods.

So far it has not been possible to recognize a lymph space around the encircled muscle fibres, such as is described with the Kuhne spindles in other somatic muscles, and in the encircled muscle

fibres the nuclei are hypo-lemmal and not central as they are in muscle spindles of the Kuhne type.

(*b*) ***Multiple or complex spirals.*** A thick, medullated fibre approaches a muscle fibre usually of large or medium size and then divides into two or more fine fibrils which, passing on either side of the muscle fibre, encircle it (Pl. 3, figs. 14–16, and Pl. 4, fig. 21) much as the thumb and fingers of a hand encircle a tube in grasping it. The fine nerve fibrils, which terminate in simplified endings in association with one or a few nuclei, pass around the muscle fibre for a varying distance, some going only a quarter way around the circumference, others continuing to pass one or more times around the muscle fibre, all intermediate stages being seen. The fibrils usually end in fine points alongside a nucleus, but whether this ending is epi- or hypolemmal has not been determined. In cross section these nerve fibrils are seen as fine, apparently interlacing, rings passing around a muscle fibre (Pl. 1, fig. 4; Pl. 3, figs. 17, 18; Pl. 4, fig. 20). Cells of the Schwann tubes can be seen adjacent to the nerve fibres and the latter are seen to terminate amongst a group of cells or near a single cell alongside the muscle fibre (Pl. 3, fig. 17).

In thick longitudinal sections stained by the Gros-Bielschowsky method both types of spiral encircling fibre can be found (simple spirals: Pl. 1, figs. 1, 2, 3, 6; Pl. 2, figs. 8, 9 and 10; complicated spirals: Pl. 3, figs. 14, 15, 16; Pl. 4, fig. 21), though not so easily as in the transverse sections, and by careful focusing the turns around the muscle fibre can be followed. Spirals are seen to be very numerous in transverse sections of the mid-zone of the muscles, around large, medium and small muscle fibres (Pl. 3, fig. 18; Pl. 4, figs. 20, 22, 23, 24, 25). A rough count of a single field showed 400 muscle fibres, thirty of these being encircled by nerve fibres. The bulk of the latter were multiple spirals, and the simple spirals were distinctly less common.

It is known that a proportion of the longitudinal fibres of the extrinsic eye muscles are encircled by other cross-striated muscle fibrils, 'Ringbinden'. Schwarz (1925) gives a description of these in normal eye muscles and Bergstrand (1938) describes them in many other muscles. Large numbers of sections have been examined to see whether there are any spiral nerve fibrils in relationship to these encircling muscle fibrils, but so far none has been found. This is not perhaps surprising, for the 'Ringbinden' have been found relatively rarely (Pl. 2, figs. 12, 13) whilst the nerve spirals are seen in great numbers.

DISCUSSION

The simple spiral nerve fibres encircling single muscle fibres, reported in this paper (Pl. 1, figs. 1, 2, 3, 6; Pl. 2, figs. 8, 9, 10), have not been found recorded in the literature dealing with human eye muscles, nor have they been found in other muscles in the body, although the annulo-spiral endings in the muscle-spindles of Kuhne bear a very remote resemblance to them. It is difficult to avoid the conclusion, on morphological grounds, that this arrangement is designed to record the cross-sectional expansion which follows the contraction of the encircled muscle fibres.

Endings of the multiple spiral type encircling single muscle fibres appear to bear some resemblance to those described by Pallot (1934) in the cat, and are undoubtedly similar to those endings seen by Hirano (1940) in longitudinal sections of human extrinsic eye muscles and believed by him to be motor, as he also considered the *terminaisons en grappe* to be. However, the motor nerve endings in all other somatic muscles are quite different in arrangement, a nerve fibre coming to the motor end-plate—a disk-like mass of cells and sarcoplasm applied to the side of a muscle fibre—and there breaking up into short finger-like processes which end between the cells of the end-plate. The ordinary motor nerve endings in the extrinsic eye muscles are similar (Pl. 2, fig. 11). No other somatic muscles have multiple nerve fibrils springing from a common trunk, and embracing a muscle fibre to end at varying points all around its periphery as is seen in the case of these multiple spiral endings. The only terminations remotely resembling these (and the resemblance is indeed fairly remote) are the 'flower spray' endings on the intrafusal fibres of muscle spindles of Kuhne (Denny-Brown, 1945). These endings in somatic muscle spindles are known to be sensory from the experimental work of Hines & Tower (1928).

The arrangement of these multiple spiral nerve fibres is well adapted to allow linear contraction and thus transverse expansion of the encircled muscle fibre to be recorded as, to use the previous analogy, a hand grasping a tube records changes in diameter.

A point which must be stressed is that endings of these types are not rare or occasional findings, but that they are present in great numbers (Pl. 3, fig. 18; Pl. 4, figs. 20, 22, 23, 24 and 25), a fact which makes it certain that they play an important part in the regulation of the contraction of these muscles.

The extraordinary abundance of nerves and nerve-endings in the extrinsic eye muscles has been commented upon by all observers, but the only nerve-endings so far demonstrated which are of undoubtedly sensory type are those resembling Golgi tendon organs at the musculo-tendinous junction. The *terminaisons en grappe*, though presumed by most observers to be sensory, have not been proved so experimentally.

Sherrington (1898, 1918), by some beautifully simple experiments and arguing logically therefrom, showed that the eye muscles must be provided with proprioceptive afferents, as are other somatic muscles, and whilst Adler (1945) believes it to be unnecessary to postulate the presence of proprioceptors he has not, in the writer's opinion, refuted Sherrington's view.

It is suggested tentatively that the spiral encircling nerve fibres described in this paper may be the extremely simplified muscle spindles which Sherrington (1897) had hoped to find, and that their discovery strengthens Sherrington's (1918) view that spatial perception is visuo-musculo-labyrinthine. It is to be hoped that electro-physiological methods may elucidate or make certain this view of their function, which at present can be no more than a hypothesis, and it is always possible that some pathological process affecting only sensory or only motor fibres may throw light on the exact site of the cells of origin of these fibres or on their function.

SUMMARY

1. Great numbers of spiral nerve endings in the mid-zone of human extrinsic eye muscles are demonstrated by means of very thick sections stained by a variety of methods, including silver techniques.

2. Simple spiral nerve endings encircling single muscle fibres have been discovered. No record of similar endings has been found in the literature.

3. Multiple spiral nerve endings, encircling single muscle fibres, are described.

4. A suggestion is made that these endings may be proprioceptors, very simple modified muscle spindles, for recording changes in stretch or contraction of the muscles.

5. It has not been possible to relate the encircling muscle fibrils found in normal extrinsic eye muscles to the encircling nerve fibres.

6. A method for still further reducing the background staining with the Gros-Bielschowsky method for staining nerve fibres is described.

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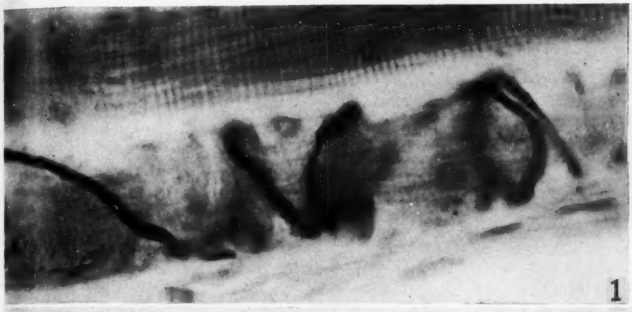
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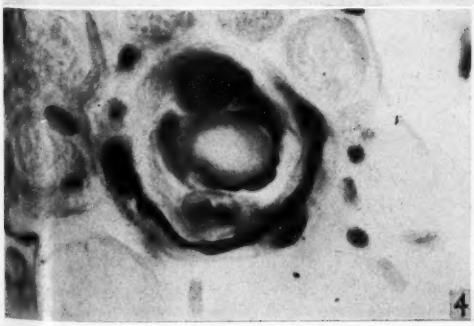
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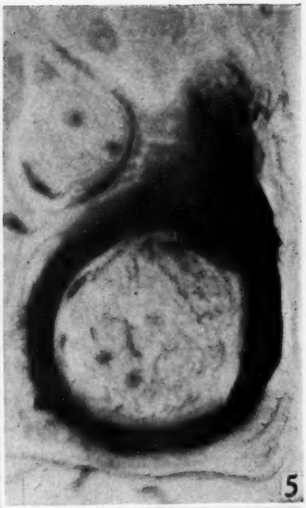
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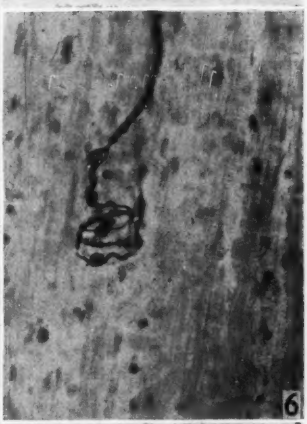
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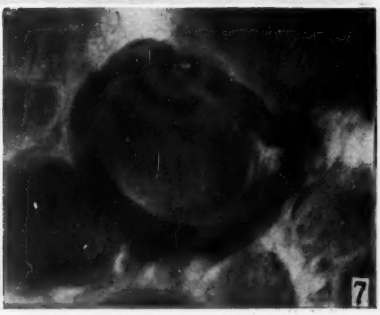
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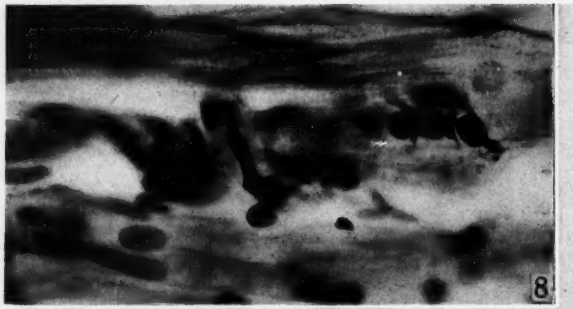
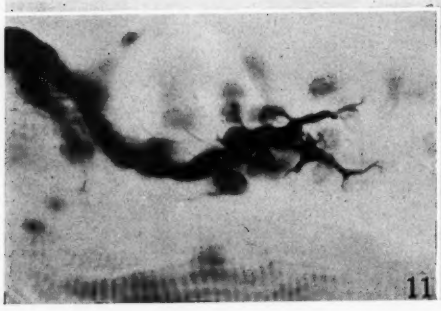
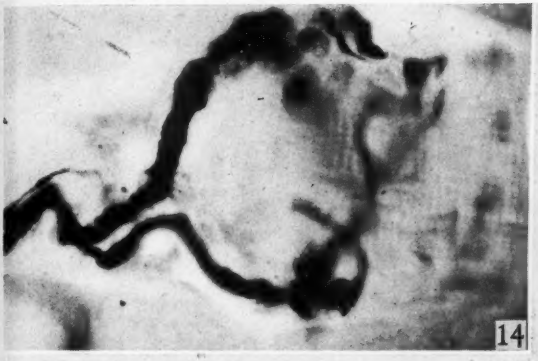




DANIEL—Spiral nerve endings in the extrinsic eye muscles of man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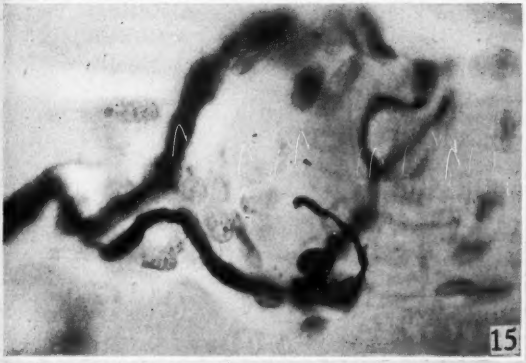
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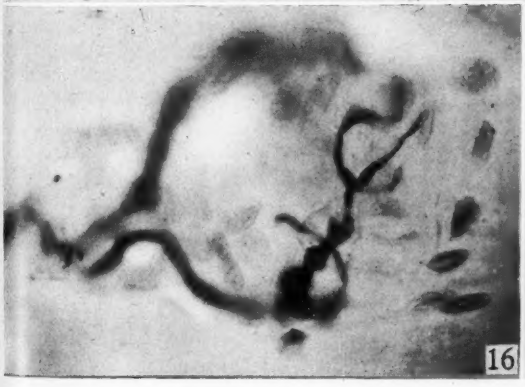
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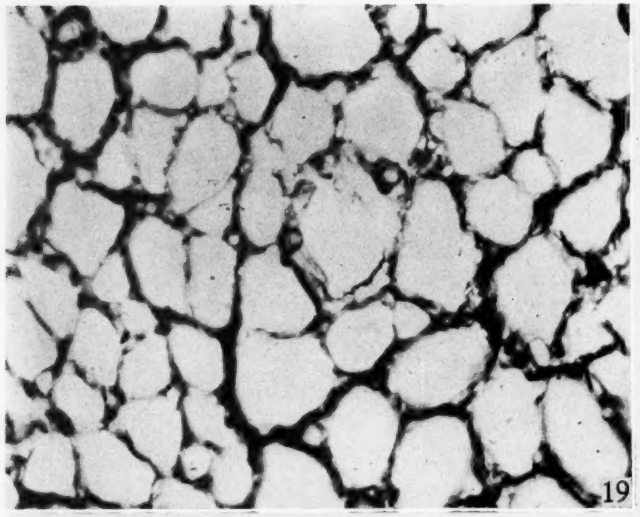
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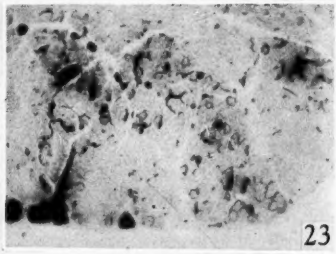
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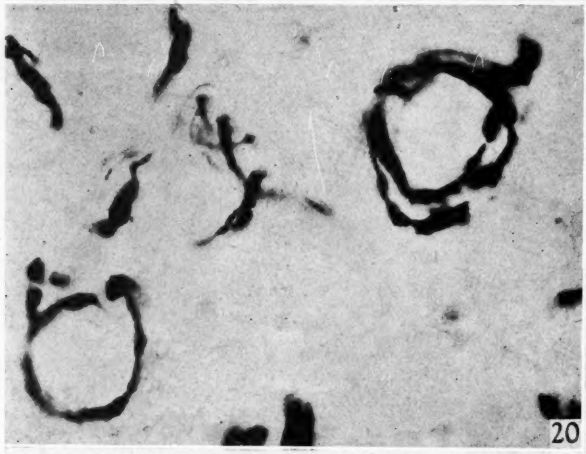
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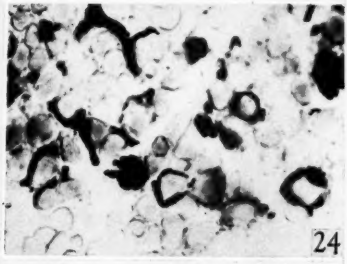
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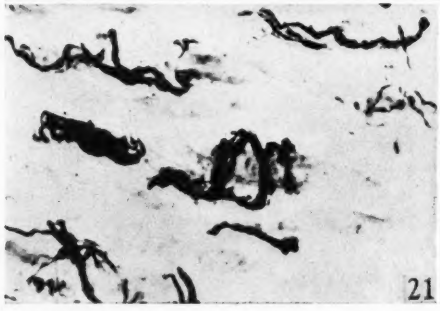
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EXPLANATION OF PLATES

Plate 1

Figs. 1–3. Longitudinal section of extrinsic eye muscle showing simple spiral nerve ending. The same spiral is photographed at three different levels, and the nerve fibre can be seen to approach from the left, to take five complete spiral turns around the muscle fibre, ending in a fine point on the right. Gros-Bielschowsky, × 720.

Fig. 4. Transverse section of extrinsic eye muscle showing complex spiral nerve ending. Two nerve fibrils can be seen passing around a rather fine muscle fibre. The outline of the sheath of Schwann and some Schwann nuclei can be seen in relation to the outer nerve fibre. Gros-Bielschowsky, × 720.

Fig. 5. Transverse section of extrinsic eye muscle showing simple spiral nerve ending. The stout myelinated nerve fibre can be seen to take one complete turn around the muscle fibre. Gros-Bielschowsky, × 1030.

Fig. 6. Longitudinal section of extrinsic eye muscle showing simple spiral nerve ending. Gros-Bielschowsky, × 320.

Fig. 7. Transverse section of extrinsic eye muscle showing simple spiral nerve ending. Essentially similar to fig. 5 but stained to show the myelin sheath, instead of being stained to show the nerve fibre. Weigert-Pal, × 820.

Plate 2

Figs. 8–10. Longitudinal section of extrinsic eye muscle showing simple spiral nerve ending. The same spiral is photographed at three different levels, and the nerve fibre can be seen to approach from the left, to take three spiral turns around the muscle fibre and to end in a series of fine finger-like processes adjacent to three nuclei (best seen in fig. 8). The outline of the Schwann sheath is seen, as are Schwann nuclei alongside the nerve fibre. Gros-Bielschowsky, × 720.

Fig. 11. Longitudinal section of extrinsic eye muscle showing motor nerve ending. Gros-Bielschowsky, × 720.

Fig. 12. Longitudinal section of extrinsic eye muscle showing encircling cross-striated muscle fibrils around a central longitudinal cross-striated muscle fibre. Mallory's P.T.A.H. × 580.

Fig. 13. Transverse section of extrinsic eye muscle showing encircling cross-striated muscle fibril around central longitudinal cross-striated muscle fibre. A small nerve trunk is seen below and to the left. Mallory's P.T.A.H. × 580.

Plate 3

Figs. 14–16. Longitudinal section of extrinsic eye muscle to show complex spiral nerve ending. The same spiral is photographed at three different levels. The stout nerve fibre can be seen at the left. It divides into two main fibrils which start to encircle the muscle fibre, each dividing later into a number of fibrils. Nuclei of the sheath of Schwann are seen and the terminal fibrils are seen to be in relationship to nuclei. Gros-Bielschowsky, × 720.

Fig. 17. Transverse section of extrinsic eye muscle to show complex spiral nerve ending. A number of apparently interlacing nerve fibrils are seen passing around the muscle fibre and some are seen to terminate in relation to nuclei on the surface of the muscle fibre. Gros-Bielschowsky, × 1030.

Fig. 18. Transverse section of extrinsic eye muscle showing two complex spiral nerve endings surrounding muscle fibres very close together. Gros-Bielschowsky, × 900.

Plate 4

Fig. 19. Transverse section of extrinsic eye muscle to show the reticulin pattern. Note how clearly the transversely cut capillaries are seen and note in the centre of the field the reticulin network around the Schwann sheath of a spiral encircling nerve fibre. Del Rio Hortega's double impregnation method for reticulin, × 720.

Fig. 20. Transverse section of extrinsic eye muscle to show two complex spiral nerve endings in one field. Gros-Bielschowsky, × 550.

Fig. 21. Longitudinal section of extrinsic eye muscle to show complex spiral nerve ending. Gros-Bielschowsky, × 230.

Figs. 22, 24, 25. Different fields from a transverse section of one extrinsic eye muscle to show the great frequency with which spiral nerve endings are found. Gros-Bielschowsky, × 140.

Fig. 23. Low-power view of transverse section of extrinsic eye muscle to show great frequency of spiral encircling nerve endings. Gros-Bielschowsky, × 40.

DEVELOPMENT OF THE EGG OF THE COW UP TO THE STAGE OF BLASTOCYST FORMATION

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The first description of the early stages of the development of the cow egg was given by Hartman, Lewis, Miller & Swett (1931). In this they described the living unsegmented egg and the two-cell stage but gave no details of the sectioned eggs. A brief account of the early development was given by one of us (W.J.H.) at a meeting of the Anatomical Society in 1941. Winters, Green & Comstock (1942) described and illustrated various stages of pre-natal development of the cow but gave few details of the early stages. Reference is made in a table by Amoroso, Griffiths & Hamilton (1942) to the rate of cleavage of the cow egg compared with that of the goat, sheep and pig.

Amongst the ungulates a detailed description of the early development has been given by Assheton (1898*a*) and Heuser & Streeter (1929) for the pig, by Assheton (1898*b*) and Clark (1934) for the sheep, and by Amoroso *et al.* (1942) for the goat. The further relevant literature in early ungulate development has been reviewed by Hartman *et al.* (1931) and Amoroso *et al.* (1942). The present communication gives an account of the early development of the cow egg from the pre-ovulation stages until the formation of the blastocyst. The material was obtained from forty-six animals which were the subject of experimental work on bovine infertility.

MATERIAL AND METHODS

The ova were collected from twenty-eight heifers and eighteen cows of various ages and breeds. They comprise two follicular oocytes removed from ovarian follicles, twenty ova obtained from normal oestrus, twenty-one obtained after oestrus induced by expression of the corpus luteum in mid-cycle, two obtained following the injection of horse pituitary extract, and one obtained following the expression of the corpus luteum and injection of pregnant mare serum (Table 1).

The animals were artificially inseminated, usually at the termination of oestrus, and were slaughtered at various intervals from 22 to 190 hr. after the end of oestrus. The end of oestrus was determined to within 4 hr. by mating tests, or was taken as 72 hr. from the time of expression of the corpus luteum, it having been found that, after removal of the corpus luteum, animals come on heat with fair regularity after approximately 60 hr. and remain in season for not more than about 12 hr. Insemination was performed in most of the cows at the presumed end of oestrus, and it was concluded that if a fertilized egg was obtained the presumption had been correct. Insemination and killing of the animals were both timed to the nearest quarter of an hour. The uterus and uterine tubes were removed within 15 min. of slaughter.

The eggs were recovered by flushing the uterine tubes or uterus with Locke's fluid into small watch-glasses. The eggs after being isolated were transferred into several changes of Locke's fluid in order to separate them from small pieces of epithelial detritus from the tube wall; they were photographed in the living state with a Vicker's projection microscope at a magnification of $\times 480$. After being photographed they were fixed in either Bouin, Flemming or Susa fixative. Most of the eggs were then embedded by the agar technique, as described by Samuel (1944), and sectioned at $7\,\mu$. Serial photographs were made of the sectioned eggs, and from these photographs wax models were constructed. Details are given of the size of the eggs before and after fixation, and also of the volume of the egg from the unsegmented stage to the eight-cell stage in the living specimen.

Calculation of size of ova. All measurements were made on photographs of living ova.

Volume of zona pellucida. The ova were assumed to be spherical, and the volume was calculated from the formula $\frac{4}{3}\pi r^3$, deriving the radius from the average measured diameter.

Diameter and volume of unsegmented ovum. Two diameters at right angles were measured and the average taken. The ova were assumed to be spherical and the volume was calculated as above.

Volume of two-cell stage. As a first approximation it may be assumed that each blastomere is an ellipsoid and so its volume can be calculated from the formula $\frac{4}{3}\pi abc$, where a, b and c are the semi-axes. By measurement it is found that b and c are equal. The total volume was obtained by addition.

Volume of four-cell stage. Where four spheres are inscribed within another, the mean diameter of the inscribed spheres is 0·9 of the radius of the circumscribing sphere.

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Table 1. *Ages and stages of development of eggs examined*

Cow no.	Age of ovum from end of oestrus (hr.)	Stage of development as shown by sections	Remarks
295	—	—	Ovarian
305	—	—	Ovarian
H.14	22	Unfertilized	Normal oestrus
I.30	24	Unfertilized	Corpus luteum expressed
95	35¼	Unfertilized	Corpus luteum expressed
437	42	Unfertilized	Corpus luteum expressed
80	46¼	Unfertilized	Normal oestrus
561	—	Not sectioned. Normal oestrus	100 mg. horse pituitary
279	48½	Maturation spindle	Corpus luteum expressed
I.39	23	2nd maturation spindle	Corpus luteum expressed
305	40	Early central pronuclei	Corpus luteum expressed
418	44½	Late centra lpronuclei	Corpus luteum expressed
I.50	50	Early eccentric pronuclei	Corpus luteum expressed
723	30½	Eccentric pronuclei	Normal oestrus
623	51¾	Pronuclei	Corpus luteum expressed 200 mg. horse pituitary
I.34	24	Pronuclei	Corpus luteum expressed
481	40	Prophase, 1st cleavage spindle	Normal oestrus
I.26	45½	Prophase, 1st cleavage spindle	Corpus luteum expressed
H.24	40	Metaphase, 1st cleavage spindle	Normal oestrus
H.15	44½	Late cleavage spindle	Normal oestrus
		Two-cell stage	
H.10	40½	Vesicular nuclei	Normal oestrus
E.87	45¾	Vesicular nuclei	Corpus luteum expressed
A.35	45	Vesicular nuclei	Corpus luteum expressed
302	96 (in uterus)	Vesicular nuclei	Corpus luteum expressed
E.D.5	45¼	Two spindles	Normal oestrus
H.23	55½	Two spindles	Normal oestrus
H.32	70	Degenerating nuclei	Pregnant mare serum—corpus luteum expressed
I.35	47	Abnormal	Corpus luteum expressed
		Three-cell stage	
620	70	One spindle, two vesicular nuclei	Corpus luteum expressed
621	44	—	Corpus luteum expressed
		Four-cell stage	
154	Unknown	Vesicular nuclei	1500 i.u. pregnant mare serum
E.92	62¾	Vesicular nuclei	Normal oestrus
H.25	66¾	Vesicular nuclei	Normal oestrus
H.5	51¼	Vesicular nuclei	Normal oestrus
H.9	43¾	Vesicular nuclei	Normal oestrus
		Six-cell stage	
625	46¼	One nucleus with spindle	Corpus luteum expressed
		Seven-cell stage	
H.13	71	Vesicular nuclei	Normal oestrus
		Eight-cell stage	
H.20	62½	—	Normal oestrus
H.6	63½	—	Normal oestrus
H.22	65	Vesicular nuclei	Normal oestrus
E.91	71	—	Corpus luteum expressed
299	72½	—	Corpus luteum expressed
282	96 (in uterus)	—	Corpus luteum expressed
		Morula stage	
I.17	72 (in uterus)	19 cells	Corpus luteum expressed
I.25	141 (in uterus)	16 cells	Normal oestrus
29	144 (in uterus)	44–48 cells	Normal oestrus
		Blastocyst stage	
H.40	190 (in uterus)	—	Normal oestrus

The diameter of the interior of the zona pellucida (the circumscribing sphere) was measured in two directions and the average taken. The mean diameters of the inscribed spheres (the blastomeres) were calculated.

The greatest and least radius of each blastomere was measured. The average of these and the mean radius was taken and the volume of each blastomere calculated from the formula $\frac{4}{3}\pi r^3$ using the average obtained as r. The total volume was obtained by addition.

Volume of eight-cell stage. The greatest and the least radius of each blastomere was measured. The average of these two was taken as the radius in each case, and the volume calculated from the formula $\frac{4}{3}\pi r^3$. The total volume was obtained by addition.

TIME OF OVULATION

Ovulation in cattle is known to occur usually after the end of oestrus. An average time of 27 hr. from the onset of oestrus is given by Hammond (1927). Calculating from this and his average figure of 17 hr. for the duration of oestrus, the average time of ovulation is 10 hr. post-oestrum. Most of the animals from which these results were obtained were heifers. Gerasimova (1938) found in 89·1 % of cases in cows that ovulation took place between 20 and 32 hr. from the beginning of heat, never earlier than 16 hr. or later than 38 hr., the average being 27 hr. 50 min. At the same time she states that the length of oestrus varied from 6 to 20 hr. and averaged 12½ hr. Therefore, ovulation occurred about 15½ hr. after the end of heat. Brewster, May & Cole (1940) found that ovulation occurred in fifty-three cows 13·57 ± 0·63 hr. post-oestrum, with a range of 2–26 hr. Nalbandov & Casida (1942) found that ovulation occurred, on the average, 14·16 hr. post-oestrum. The latter workers found also that in two out of seventy-two oestral periods studied in twenty-two cows ovulation was delayed for from 47 to 104 hr.

There is thus general agreement that ovulation occurs at from about 10 to 15½ hr. post-oestrum, the shorter time in heifers, and the longer in cows.

OBSERVATIONS

Ovarian oocyte

Two oocytes, with their surrounding corona radiata cells, were removed from large ovarian follicles and after fixation were sectioned.

The first oocyte was recovered from an almost mature follicle of a cow (no. 295); after sectioning it was found to have a mean diameter of 94 μ.

The second oocyte (Pl. 1, fig. 1) was recovered from a heifer (no. 305) that had been in oestrus 40 hr. previously; after sectioning, this oocyte had a mean diameter of 79 μ. An unsegmented egg at the pronuclear stage was also recovered from the uterine tube of this animal.

In each of these oocytes the cytoplasm was finely granular in the centre of the egg, but more coarsely granular at the periphery. In each there was a large vesicular nucleus with a distinct nuclear membrane and scattered nuclear chromatin.

Unsegmented ovum

In our material there are eighteen unsegmented eggs, some of which are unfertilized while others are at various pronuclear and spindle stages (see Table 1).

The times of ovulation, as already stated, range from 10 to 15½ hr. after the termination of oestrus. Six eggs were obtained from cows which had ovulated after a normal oestrous cycle; eleven eggs were procured from animals in which oestrus had been induced by the expression of the corpus luteum of the previous cycle; in one animal of the latter group horse pituitary extract was administered (see Table 1).

Single-cell stage

The living ovum

Owing to the presence of a large number of fatty globules in the vitellus it is not possible by an examination of the living egg to state whether or not the egg has been fertilized. In one specimen only (no. I.30), obtained 24 hr. post-oestrum, were corona radiata cells found attached to the zona pellucida. This specimen, as subsequent histological examination showed, was in the early stages of degeneration, as many scattered vacuoles were found in the cytoplasm and the chromatin material was no longer recognizable.

The zona pellucida appears as a homogeneous and structureless membrane with an average thickness of 16·5 μ. In it, as in the eggs from most of the animals that had been inseminated, many sperms were seen while the egg was alive, and their presence was confirmed by subsequent examination of the sections.

In the recently ovulated unfertilized egg the vitellus completely fills the zonal cavity (Pl. 1, fig. 2). In all of the fertilized eggs there is a distinct perivitelline space and hence the volume of the vitellus is less than that of the zonal cavity (Table 2). The presence of the space, however, is not a criterion of fertilization since a space is also present in degenerating eggs. Polar bodies are only occasionally seen in the perivitelline space of living eggs (Pl. 1, fig. 3). The vitellus is composed of a large number of fine, highly refractile granules evenly distributed in the cytoplasm, there being no evidence of polarity in the normal egg. In many eggs, which subsequent histological examination showed to be degenerating, there was a crescentic area of varying extent that was devoid of granules.

The fixed ovum

Agar whole mounts

After embedding in agar, and dehydration, many of the eggs were photographed and examined as whole mounts in methyl benzoate. It will be seen by comparing Pl. 1, figs. 3 and 4 that the egg has undergone a considerable shrinkage. The fatty material has been dissolved by the alcohol and the cytoplasm has assumed a granular appearance. In many of the whole mounts it was possible to recognize the nuclear structure of the egg (Pl. 1, fig. 4).

Sectioned material

Two eggs showing a maturation spindle were recovered from cows (nos. I.39 and 279) at 23 and 48½ hr. post-oestrum. The corpus luteum of the previous cycle in each animal was expressed 60 hr. previous to the onset of oestrus. Both animals were artificially inseminated at the end of oestrus but the eggs were unfertilized.

As will be seen from Table 1 there are several eggs at the pronuclear stage. The youngest pronuclear stage (I.34) was obtained 24 hr., the oldest (no. 623) 51¾ hr. post-oestrum. This last egg (no. 623) showed degenerative changes and it seems probable that it would not have developed further. Typical eccentrically placed pronuclei are seen in Pl. 1, figs. 5 and 6. This egg was obtained from a cow (no. 723) 30½ hr. post-oestrum. Centrally placed pronuclei obtained from a cow (no. 418) 44½ hr. post-oestrum are shown in Pl. 2, fig. 7; at this stage the pronuclei are large and vesicular. In none of

Table 2. *Volume of vitellus and zonal cavity in living unsegmented and segmented eggs*

Cow no.	External diameter of zona pellucida (μ)	External volume of zona pellucida (cu.μ)	Internal diameter of zona pellucida (μ)	Internal volume of zona pellucida (cu.μ)	Thickness of zona pellucida (μ)	Diameter of vitellus (μ)	Volume of vitellus (cu.μ)
437	168·7	2,515,000	137·5	1,361,000	15·6	112·5	745,500
443	170·8	2,610,000	137·5	1,361,000	16·65	118·8	878,300
279	181·25	3,118,000	148·0	1,697,000	16·625	131·25	1,183,500
I.50	172·9	2,708,000	141·66	1,489,000	15·62	120·8	984,600
481	172·9	2,708,000	131·25	1,183,500	20·825	116·6	830,400
I.26	160·0	2,145,000	135·4	1,300,000	13·3	116·6	830,400
H.24	168·7	2,515,000	137·5	1,361,000	15·6	120·8	984,600
			Two-cell stage				
I.35	164·6	2,335,000	133·33	1,242,000	15·635	—	—
E.87	181·25	3,118,000	150·0	1,768,000	15·625	—	1,855,000
A.35	170·8	2,610,000	143·75	1,556,000	13·525	—	644,000
302	166·66	2,424,000	137·5	1,361,000	14·58	—	1,127,000
E.D.5	175·0	2,806,000	139·6	1,424,000	17·7	—	661,000
			Three-cell stage				
620	168·7	2,515,000	139·6	1,424,000	14·6	—	1,044,000
			Four-cell stage				
H.5	177·0	2,903,000	143·75	1,556,000	16·625	—	743,000
H.9	162·5	2,249,000	139·6	1,424,000	11·45	—	714,000
H.25	175·0	2,806,000	139·6	1,424,000	17·7	—	—
			Six-cell stage				
625	158·33	2,078,000	129·16	1,128,000	14·535	—	—
			Seven-cell stage				
H.13	164·6	2,335,000	135·4	1,300,000	14·6	—	—
			Eight-cell stage				
H.20	160·0	2,145,000	135·4	1,300,000	12·3	—	—
H.6	160·0	2,145,000	139·6	1,424,000	10·2	—	680,000
H.22	164·6	2,335,000	141·66	1,489,000	11·47	—	—
E.91	177·0	2,903,000	148·0	1,697,000	14·5	—	—
299	168·7	2,515,000	141·66	1,489,000	13·52	—	803,000
282	170·8	2,610,000	141·66	1,489,000	14·57	—	—
			Morula stage				
I.17	168·7	2,515,000	139·6	1,424,000	14·55	—	—
I.25	168·7	2,515,000	143·75	1,556,000	12·475	—	—
29	164·6	2,335,000	137·5	1,361,000	13·55	—	—
			Blastocyst stage				
H.40	168·7	2,515,000	137·5	1,361,000	15·6	—	—

the stages in our collection is it possible to state which is the male and which is the female pronucleus.

First cleavage spindles (Pl. 2, fig. 8) were obtained at 40 hr. in cows (nos. 481 and H.24) at 44½ hr. (H.15) and at 45½ hr. (I.26) post-oestrum; the youngest was obtained after normal oestrus and the oldest after expression of the corpus luteum.

Two-cell stage

There are eight eggs at this stage of development. A two-cell stage with vesicular nuclei was recovered from the uterine tube 40½ hr. post-oestrum (H.10). This is the earliest time at which a two-cell stage was found. The latest time at which a normal two-cell stage was recovered following normal oestrus was 55½ hr. (no. H.23). Each blastomere in this egg shows a spindle of division and these are arranged approximately at right angles to each other. A two-cell stage was recovered 96 hr. after oestrus induced by the expression of the corpus luteum. This egg was undergoing degenerative changes, many vacuoles being present in the cytoplasm.

In the normal living egg (Pl. 2, fig. 9), in the agar preparation (Pl. 2, fig. 10), and in the histological specimen (Pl. 2, fig. 11), the cytoplasm is similar in appearance to that of the unsegmented egg (Pl. 1, figs. 3, 4 and Pl. 2, fig. 8). The blastomeres at the two-cell stage are similar in appearance but show slight differences in their volume (see Table 3).

Three-cell stage

There are two eggs at this stage, one at 44 hr. and the other at 70 hr. post-oestrum. In each cow the corpus luteum of the previous cycle had been expressed. One of the cells in each of the specimens was much larger than the other cells. In the 70 hr. ovum (no. 620) the large cell showed a spindle of division.

Four-cell stage

Five eggs at this stage of development were obtained from five cows at times ranging from 43¾ to 66¾ hr. post-oestrum. The eggs, with one exception (no. 154), were obtained following a normal oestrous period. Egg 154 was obtained from an animal which received 1500 i.u. of pregnant mare serum 1 day before the corpus luteum was expressed. The animal was slaughtered 4 days after the expression of the corpus luteum; the precise time of mating was not known. Cow E.92 was inseminated 13½ hr. post-oestrum, whilst the other three cows were inseminated at the end of oestrus.

The blastomeres in all of the eggs were arranged in pairs so as to form a regular cross, i.e. in a tetrahedral formation (Pl. 2, fig. 12 and Pl. 3, fig. 13). Except for slight differences in size the blastomeres both in the living egg and after sectioning were similar in appearance. The cytoplasm (Pl. 3, fig. 14) was essentially similar to that of the unsegmented egg. The nuclei of all the blastomeres were in the

Table 3. *Calculated volumes of the blastomeres of living eggs from two- to eight-cell stage*

Cow no.	Volumes of individual blastomeres (living ova) (cu.μ)								Total volume (cu.μ)
				Two-cell stage					
A.35	339,000 53%	305,000 47%							644,000
E.D.5	334,000 51%	327,000 49%							661,000
E.87	1,023,000 55%	832,000 45%							1,855,000
302	666,000 59%	461,000 41%							1,127,000
				Three-cell stage					
620	524,000 50%	260,000 25%	260,000 25%						1,044,000
				Four-cell stage					
H.9	239,000 34%	187,000 26%	144,000 20%	144,000 20%					714,000
H.5	268,000 36%	187,000 26%	144,000 19%	144,000 19%					743,000
				Eight-cell stage					
299	144,000 18%	108,000 13·5%	105,000 13%	102,000 12·5%	92,000 11·5%	87,000 11%	87,000 11%	78,000 9·5%	803,000
H.6	102,000 15%	92,000 13·4%	87,000 12·8%	87,000 12·8%	82,000 12%	82,000 12%	74,000 11%	74,000 11%	680,000

resting state. The measurements of the volumes of the cells are given in Table 3.

Six-, seven- and eight-cell stages

At these stages of development there are eight specimens which were obtained from eight cows.

Egg 625 is at the six-cell stage and was recovered 46¼ hr. post-oestrum following the expression of the corpus luteum. Two of the cells, one with a spindle and the other with a resting nucleus, are much larger than the other four cells. The disposition of the cells was such that accurate measurements of them could not be made in the living specimen.

Egg H.13 is at the seven-cell stage and it was recovered 71 hr. after the termination of a normal oestrus. In this egg one cell is larger than the others (Pl. 3, fig. 15). All the nuclei are in the resting condition.

The remaining six eggs, at the eight-cell stage, were recovered from six cows at times ranging from 62½ to 96 hr. post-oestrum. Three of the eggs (nos. H.20, H.6 and H.22) were recovered following normal oestrus; the three other eggs (nos. 282, 299 and E.91) were recovered from cows after the expression of the corpus luteum. All the eggs, with the exception of no. 282, were from the uterine tube; this latter egg was recovered from the uterus at 96 hr. post-oestrum.

The cells at the eight-cell stage are approximately spherical in shape and are surprisingly uniform in size (Pl. 3, figs. 16, 17, and Table 3). The blastomeres are closely packed in the zonal space and are arranged as two groups of four cells, which interdigitate with one another. Many sperms are still present in the zona pellucida. In the sectional material the cytoplasm was similar to that of earlier stages of development (Pl. 3, figs. 18, 19). There is as yet no microscopical evidence of differentiation among the cells.

Morula stage

There are three eggs at this stage of development.

A morula with nineteen cells was recovered from the uterine tube of a cow (no. I.17) 72 hr. post-oestrum following the expression of the corpus luteum. In the living state the blastomeres were seen to be approximately of equal size (Pl. 4, fig. 20) and this was confirmed when the sectioned egg was examined. The egg when cut was present in seventeen sections of 8 μ giving it a maximum diameter of 126 μ. One blastomere is centrally placed, the other cells being arranged around it (Pl. 4, figs. 21, 22). In the fixed and sectioned egg all the blastomeres have resting nuclei and the cytoplasm of the cells shows no qualitative differences.

A second morula of sixteen cells was recovered from the uterine cavity of a cow (no. I.25) killed 141 hr. after normal oestrus. In the living egg (Pl. 4, fig. 23) slight differences in the size of the cells could be readily appreciated. The egg, which was cut at 7 μ, was present in fifteen sections giving it a diameter of 105 μ. A single blastomere is again centrally placed as in the previous morula. In the fixed and sectioned egg all the cells have resting nuclei and are essentially similar in appearance. The differences in size and form of the blastomeres at the morula stage as described by Amoroso *et al.* (1942) for the goat are not manifest in the egg of the cow.

The third morula was obtained from the uterine cavity of a cow (no. 29) killed 144 hr. after normal oestrus. In the living state, owing to the number of cells, the differences in the size of the blastomeres could not be readily appreciated. Irregularly arranged intercellular spaces, the forerunners of the blastocyst cavity, are present between the outer layer of cells and those in the interior of the egg (Pl. 4, figs. 24, 25).

The morula stages at our disposal give no indication as to how the centrally placed cell comes to occupy its position.

Blastocyst stage

An egg at this stage was recovered from the uterine cavity of a cow (no. H.40) killed 190 hr. after normal oestrus. The appearance of the egg in the living state is shown in Pl. 5, fig. 26. It will be seen that the cells have shrunk from the inner aspect of the zona pellucida leaving an extensive perivitelline space. After fixation and clearing the blastocyst cavity can readily be recognized (Pl. 5, fig. 27).

On section the blastocyst is found to consist of an outer layer of cuboidal or irregularly polygonal cells and an inner mass; the latter is in close relationship with the cells which cover it. There is, as yet, no visible difference between the covering cells and the inner cell mass, nor is there any segregation of the cells of the inner mass into embryonic ectoderm and endoderm (Pl. 5, figs. 28–30).

COMMENTS AND COMPARISONS

Zona pellucida

The zona pellucida of the cow egg has an appearance similar to that of other mammalian eggs. It persists at least until the blastocyst stage at 190 hr. post-oestrum.

Cumulus cells

In contrast to many mammalian eggs, e.g. guinea-pig (Squier, 1932), rat (Gilchrist & Pincus, 1932), and ferret (Hamilton, 1934), the cumulus cells in the cow disappear soon after ovulation. In only one specimen, recovered 24 hr. post-oestrum, were a few cumulus cells attached to the zona pellucida.

An unfertilized egg recovered as early as 22 hr. post-oestrum showed no cumulus cells. The examination of eggs removed from large ovarian follicles shows that there is a well-organized layer of cumulus cells. Webster (1921) refers to cumulus cells as being present in the large follicles. The cells persist for not more than 9–14 hr. after ovulation and in most eggs for even a shorter period.

Of the ungulates so far examined, cumulus cells, soon after ovulation, are absent on the eggs of the sheep (Assheton, 1898*a*; McKenzie & Allen, 1933; Clark, 1934; and McKenzie & Terrill, 1937), the cow (Hartman *et al.* 1931; and Evans & Miller, 1935), the pig (Assheton, 1898*b*; Corner & Amsbaugh, 1917; and Heuser & Streeter, 1929), the horse (Amoroso *et al.* 1939; and Hamilton & Day, 1945), and the goat (Amoroso *et al.* 1942). Whether these ungulates show a marked loosening of the cumulus cells such as is described by Lewis & Hartman (1941) in the pre-ovulatory stages in the monkey, we have no evidence to present. We can offer no explanation for the rapid disappearance of the cells, but suggest that it may be a characteristic of the ungulates.

There can be little doubt that the presence of living spermatozoa may assist in the separation of the cells, but that they are not essential is shown by the fact that the cells are absent in unfertilized as well as in fertilized eggs. From the large number of spermatozoa found in the zona pellucida of many fertilized eggs, it appears that large numbers of them surround the ovum in its passage down the uterine tube. Pincus (1930) and Pincus & Enzmann (1932) have shown that in the rabbit and rat respectively when eggs with attached cumulus cells are placed *in vitro* with living sperm suspensions the cells are rapidly dispersed, but this does not occur in sperm-free media. Yamane (1930) believes that the dispersion is due to a proteolytic enzyme in the spermatozoa. For details of the effect of sperm suspensions on maturation, etc., the work of Pincus & Enzmann (1935) should be consulted.

Cytoplasm of the ovum

There are striking differences in the appearances of living eggs of different mammals; even among the eggs of a single genus, differences are apparent. In some, e.g. the cow, the cytoplasm of the egg is made up of many small discrete fatty globules which are closely packed together. In others, e.g. the mouse and the golden hamster, the fatty globules are absent.

The nucleus cannot be seen in the living egg of the former group, whereas such structures as the nucleus and even the sperm head and tail may be easily recognized in the latter. Amongst the ungulates, the eggs of the sheep and goat are strikingly similar in appearance and contain much less fatty material than those of the cow, whilst the eggs of the pig and horse contain much more fatty material. There is no evidence of polarity in the cow egg and in this respect it is similar to the eggs of the sheep, goat, pig and horse.

Location of the eggs

No attempt has been made to determine the precise location of the eggs during their passage through the uterine tube. The times at which the different stages were recovered are shown in Table 4.

The earliest time at which an egg was found in the uterus was 96 hr. post-oestrum—a two-cell stage from cow 302 and an eight-cell stage from cow 282. For the eight-cell stage the time of ovulation was estimated to be between 10 and 15½ hr. post-oestrum, which means that the time taken for the tubal journey of this egg was from 80½ to 86 hr. The time interval between insemination and the entry of the egg into the uterus is given in Table 4 for a number of mammals. This table, however, does not give a true estimation of the time taken for the tubal transit since in some forms (e.g. mouse) ovulation occurs any time during oestrus, in others (e.g. rabbit) shortly after copulation, while in others (e.g. ferret) not until 31½–53½ hr. after copulation. If due allowance is made for this variation in the times of ovulation in relation to oestrus and insemination, the tubal journey takes about 3–3½ days in most mammals with the exception of the opossum, as estimated by Hartman (1939).

Volume of the vitellus

A review of the size of the mammalian egg was given by Hartman (1929). At that time relatively few accurate observations had been made of the size of the living egg, hence estimations of the size of many eggs were deduced from calculations made on fixed specimens.

Since that date a number of papers (Gregory, 1930; Gilchrist & Pincus, 1932; Squier, 1932; Lewis & Hartman, 1933; Clark, 1934; Lewis & Wright, 1935; and Amoroso *et al.* 1942) have appeared. These publications give measurements of the living eggs of different species. Many of the authors have given the diameters of the zona pellucida and vitellus only, whilst a few have calculated the volume of the egg during cleavage. The vitellus of the cow egg has a diameter which is approximately 1½ times greater than that of the mouse and a volume that is approximately 4 times greater than that of the mouse. Lewis & Wright (1935) point out that 'the difference in volume is far more striking and probably more significant'.

In the present investigation we have calculated, in the living egg, the volume of the vitellus in the unsegmented egg, and of the blastomeres up to the eight-cell stage in the living egg and also in the fixed specimen. The examination of the living specimen

Table 4. *Stages of development in hours of different species*

Type	Observer	Estimated time of ovulation	1-cell	2-cell	3–4-cell	5–8-cell	9–16-cell	Morula	Blastocyst	Time and stage at which egg reaches uterus	Total gestation period (days)
Ungulate:											
Cow	Present series	10–15·5 hr. post-oestrum	23–51·75	40·5–55·5	44–65·75	46·25–96	71–141	144	190	96 (8–16-cell)	284
Cow	Hartman *et al.*	—	—	48	—	—	—	—	—	—	—
Cow	Winters *et al.*	Post-oestrum	34	50–62	—	62–64	110	134	182	110 (16-cell)	284
Goat	Amoroso *et al.*	—	30·5	30·5–48	60	85	98	120–140	158	98 (10–13-cell)	150
Sheep	Clark	Late in oestrus	0–38·75	38–39	42	44	65–77	96	113·5–138·75	77–96 (16-cell)	150
Pig	Heuser & Streeter	—	0–51	51–66	66–72	90–110	—	110–114	114	75 (4-cell)	112
Carnivora:											
Ferret	Hamilton	31·5 hr. after copulation	31·5–53·5	—	64–72	64–116·5	74–120	120–146	146–264	120–140 (up to 32-cell)	42
Primate:											
Macacus rhesus	Lewis & Hartman	Determined by bimanual palpation	—	0–24	24–36	36–48	48–72	72–96	—	96 (16-cell)	164
Rodent:											
Mouse	Lewis & Wright	Any time during oestrus	0–24	24–38	38–50	50–64	60–70	68–80	74–82	72 (morula)	19–21
Mouse	Sobotta	—	0–24	24–38	50	60	70	—	—	—	—
Rat	Huber	—	0–24	42–70	63–73	89	89–96	—	—	—	—
Rat	Gilchrist & Pincus	As early as 8·5 hr. after copulation	8·5–27	27–44	60–85	71–95	—	—	—	—	22
Rat	Macdonald & Long	—	12–20 (15)	37–61 (45)	57–85 (65)	64–87 (79)	84–92 (90)	—	105–109 (107)	—	—
Rabbit	Gregory	—	Up to 22	22–26	26–32	32–40	40–47	47–68	68–76	70 (blastocyst)	30–32
Rabbit	Assheton	—	12–24	24–28	28	—	—	48	75–96	—	—
Rabbit	Pincus	10 hr. after copulation	11–21	21–24	—	—	—	—	—	—	—
Guinea-pig	Squier	After copulation	3–30	30–35	30–75	80	—	100–115	115–140	80–85 (8-cell)	63

is not vitiated by the different amount of shrinkage which occurs in fixed material (Table 6). We have compared (see Table 5) the volumes so obtained with those given by other investigators or estimated by us from photographs of living eggs or from data given in the articles quoted. Data and comparisons of a similar nature derived from models and other calculations have been given by other investigators for fixed eggs. It has not, however, been possible to estimate the volume of the different blastomeres beyond the eight-cell stage as the cells overlap in the photographs.

Cleavage

The differences in the rates of cleavage of the eggs of different species have been commented upon by many investigators and are set out in tabular form (Table 4). There are also variations in the time at which cytological differentiation becomes manifest amongst the blastomeres, and in the relative sizes of the blastomeres in different species during the early cleavage stages (see Amoroso *et al.* 1942 for literature).

In the cow egg there are not the striking differences in the size of blastomeres during the early stages of cleavage nor is there the early segregation of the blastomeres into larger, more slowly dividing cells, and smaller, more actively dividing cells, as described by Heuser & Streeter (1929) in the pig and to a lesser extent by Amoroso *et al.* (1942) in the goat. In the morula stages the blastomeres show only slight differences in their size and are cytologically similar. This relative lateness of differentiation in the egg of the cow may be associated with the longer gestation period compared with that of the pig and goat (see Table 4).

SUMMARY

1. An account is given of the cleavage stages of the egg of the cow.

2. The rate of cleavage is discussed and compared with that of other mammalian eggs.

3. The diameters of the different eggs and of the volumes of the blastomeres during cleavage as far as the eight-cell stage are given.

4. The shrinkage undergone by the eggs as the result of fixation is estimated.

5. The time taken by the egg to traverse the uterine tube is 96 hr.

6. Histological differentiation is not apparent in the late morula and early blastocyst stage.

Table 5. *Volumes of vitellus in different species*

Type	Observer	Average internal diameter of zona pellucida (μ)	Volume of ovarian ovum (cu.μ)	Average diameter of vitellus (μ)	Average volume of vitellus in cu.μ				
					1-cell	2-cell	4-cell	8-cell	Morula
Ungulate:									
Cow	Present series	138·4	—	120	919,500	1,071,750	729,000	741,500	—
Cow	Hartman *et al.*	143	1,537,000	120	907,200	—	—	—	—
		—	1,298,000	—	—	679,000	—	—	—
		135	1,600,000	—	—	—	—	—	—
Goat	Amoroso *et al.*	145·3	1,615,000	132	1,204,000	—	—	—	—
Sheep	Calculated from Clark	147	1,663,000	—	1,056,000	919,000	712,200	615,700	—
Pig	Heuser & Streeter	130	1,150,400	111	716,090	—	—	—	—
Primate: (Macacus rhesus)	Lewis & Hartman	125–143	1,000,000–1,633,000	140 (3 eggs)	549,000 (3 eggs)	562,000	527,000	546,000	—
Rodent:									
Mouse	Lewis & Wright	87·8	254,000	71·6	192,000	158,000	162,000	138,000	219,000
Ferret	Hamilton	153	1,876,000	124	1,003,000	881,249	842,000	737,000	—

Table 6. *Volumes of vitellus in living and fixed ova*

Cow no.	Volume of vitellus of living ovum (cu.μ)	Volume of vitellus of fixed ovum (cu.μ)	Loss during fixation %
A.35	644,000	430,000	33
E.D.5	661,000	430,000	36
H.9	714.000	460.000	35
H.5	743,000	570,000	24
299	803,000	290,000	64
H.20	680,000	360,000	47
H.40	—	80,000	—

It is a pleasure to record our indebtedness to those who have given of their services in many different ways. We wish to thank Dr J. S. Paterson, of the Institute of Animal Pathology, Cambridge, for giving us access to some of the animals used in this investigation. We are indebted to Mr W. S. Mansfield, Director of the University Farm, Cambridge, for providing accommodation for some of the animals. Part of the work was carried out by one of us (J.A.L.) while holding the Miss Aleen Cust

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Photograph of a section of an oocyte recovered from a large ovarian follicle of a heifer (no. 305) killed 72 hr. post-oestrum. × *c.* 416.

Fig. 2. Photograph of a living recently ovulated unfertilized ovum recovered from a cow (no. 107). × *c.* 280.

Fig. 3. Photograph of a living fertilized ovum recovered from a cow (no. 481) killed 40 hr. post-oestrum. A polar body is visible. In the zona pellucida there are many sperms. × *c.* 384.

Fig. 4. Photograph of unsegmented ovum, after fixation and clearing, recovered from a cow (no. 126) killed 45·5 hr. post-oestrum. × *c.* 384.

Figs. 5, 6. Photographs of two adjacent sections of a zygote with eccentrically placed pronuclei. The cow (no. 723) was killed 30·5 hr. post-oestrum. × *c.* 416.

Plate 2

Fig. 7. Photograph of a section of a zygote with centrally placed pronuclei recovered from a cow (no. 418) killed 44·5 hr. post-oestrum. × *c.* 512.

Fig. 8. Photograph of a section of a zygote with first cleavage spindle recovered from a cow (no. 481) killed 40 hr. post-oestrum (cp. with Pl. 1, fig. 4). × *c.* 416.

Fig. 9. Photograph of a living two-cell stage recovered from a cow (no. E.D.5) killed 45·5 hr. post-oestrum. × *c.* 384.

Fig. 10. Photograph of a two-cell stage, after fixation and clearing, recovered from a cow (no. H.23) killed 55·5 hr. post-oestrum. × *c.* 384.

Fig. 11. Photograph of a section of the same two-cell stage as shown in fig. 10. The nuclei are at the prophase. × *c.* 416.

Fig. 12. Photograph of a living four-cell stage recovered from a cow (no. H.9) killed 43·75 hr. post-oestrum. × *c.* 384.

Plate 3

Fig. 13. Photograph of a four-cell stage after fixation and clearing recovered from a cow (no. H.5) killed 51·5 hr. post-oestrum. × *c.* 416.

Fig. 14. Photograph of a section of the same four-cell stage as shown on fig. 13. Many sperm heads are seen in the zona pellucida. × *c.* 416.

Fig. 15. Photograph of a seven-cell stage, after fixation and clearing, recovered from a cow (no. H.13) killed 71 hr. post-oestrum. × *c.* 384.

Fig. 16. Photograph of a living eight-cell stage recovered from a cow (no. H.20) killed 62·5 hr. post-oestrum. × *c.* 384.

Fig. 17. Photograph of the same egg as in fig. 16 after fixation and clearing. × *c.* 384.

Figs. 18, 19. Photographs of the fifth and ninth sections of the same egg as in figs. 16 and 17. × *c.* 416.

PLATE 4

Fig. 20. Photograph of a living morular stage (19 cells) recovered from a cow (no. I.17) killed 72 hr. post-oestrum. × *c.* 384.

Figs. 21, 22. Photographs of the tenth and eleventh sections of the same egg as in fig. 20. × *c.* 416.

Fig. 23. Photograph of a living morular stage (16 cells) recovered from a cow (no. I.25) killed 141 hr. post-oestrum. × *c.* 384.

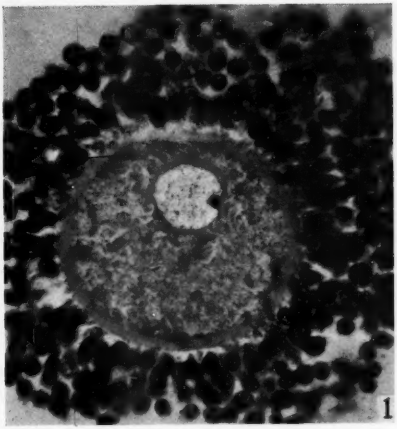
Figs. 24, 25. Photographs of the eighth and ninth sections of an egg recovered from a cow (no. 29) killed 144 hr. post-oestrum. × *c.* 416.

PLATE 5

Fig. 26. Photograph of a living blastocyst recovered from a cow (no. H.40) killed 190 hr. post-oestrum. × *c.* 384.

Fig. 27. Photograph of the same blastocyst as in fig. 26 after fixation and clearing. The blastocyst cavity can be recognized. × *c.* 384.

Figs. 28–30. Photographs of the eighth, ninth and tenth sections of the blastocyst shown in fig. 26. × *c.* 416.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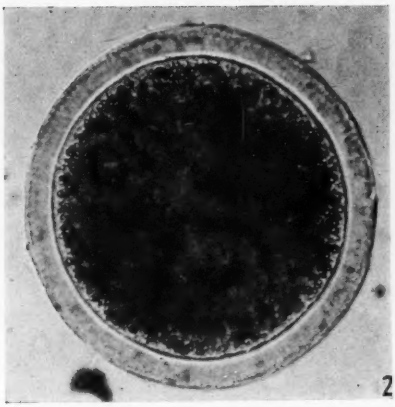
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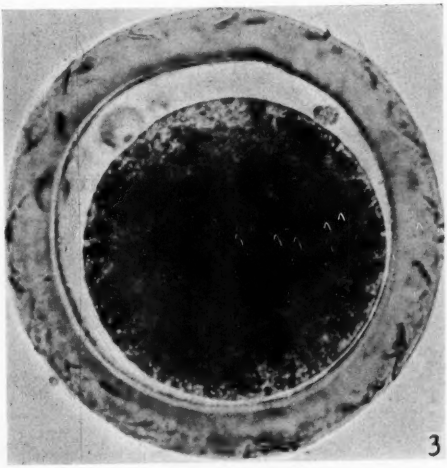
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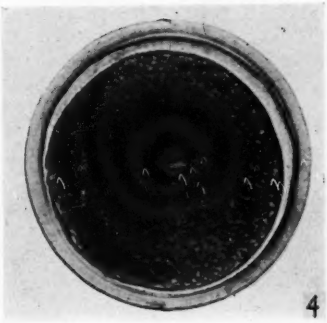
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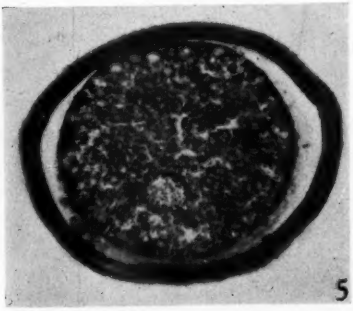
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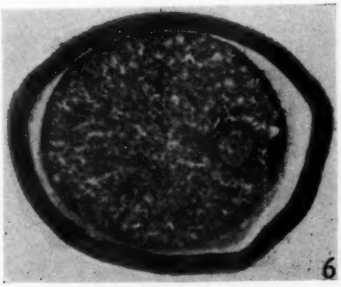
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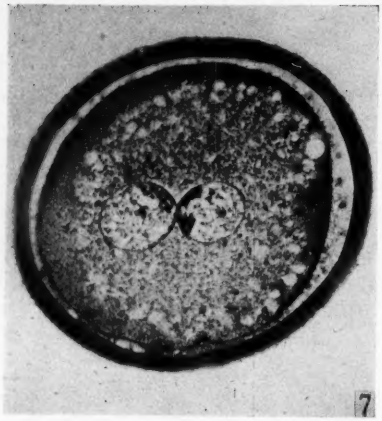
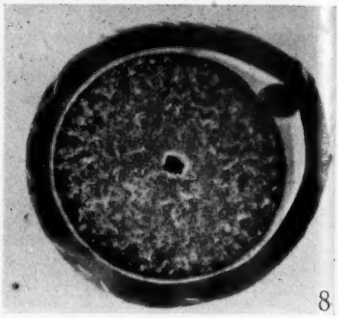
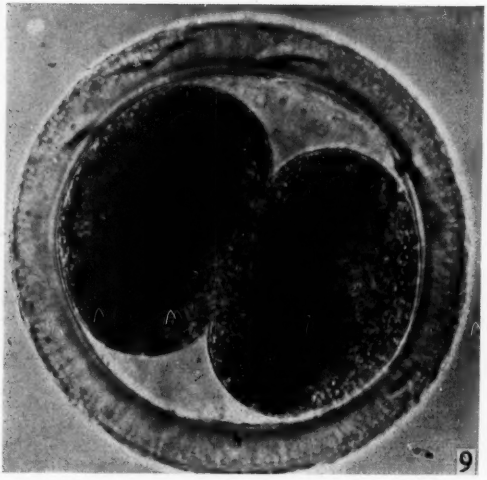
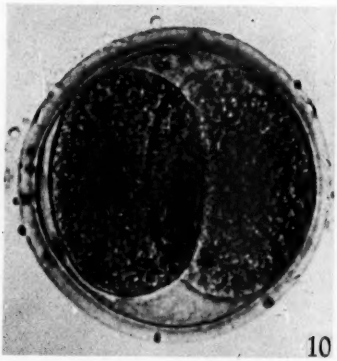
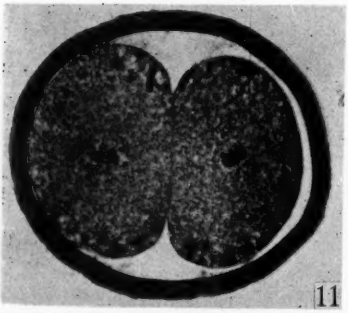
HAMILTON AND LAING—DEVELOPMENT OF THE EGG OF THE COW UP TO THE STAGE OF BLASTOCYST FORMATION

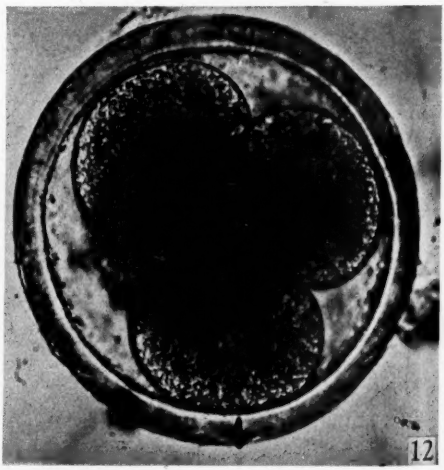




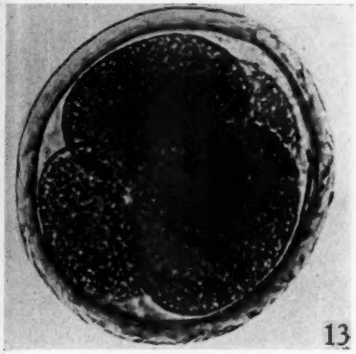








HAMILTON AND LAING—DEVELOPMENT OF THE EGG OF THE COW UP TO THE STAGE OF BLASTOCYST FORMATION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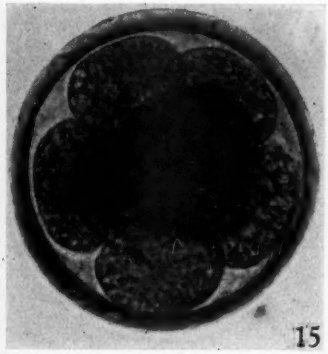
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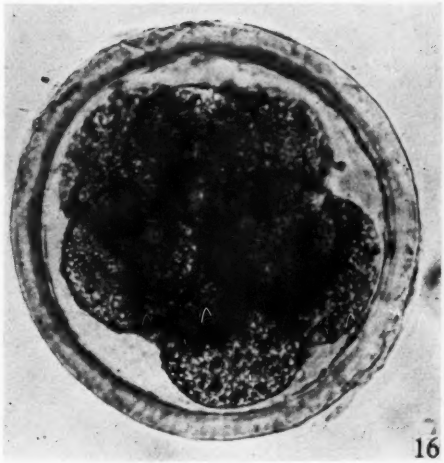
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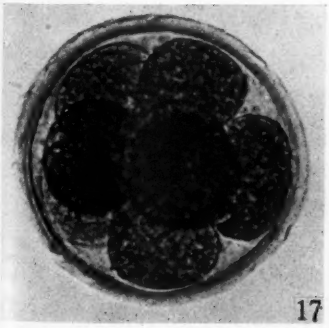
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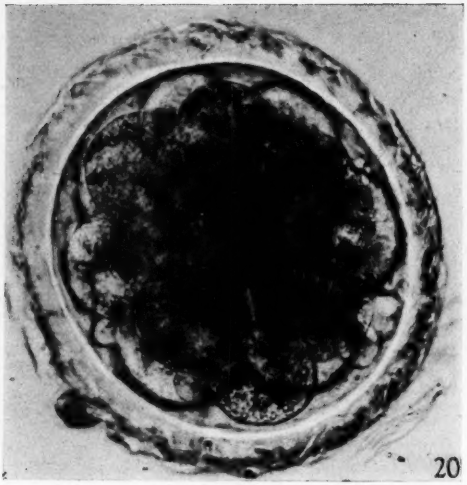

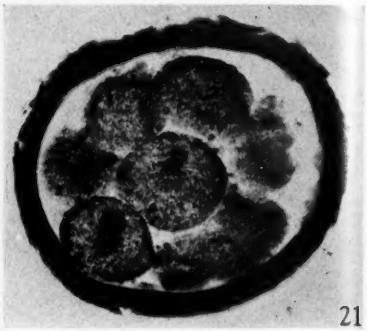

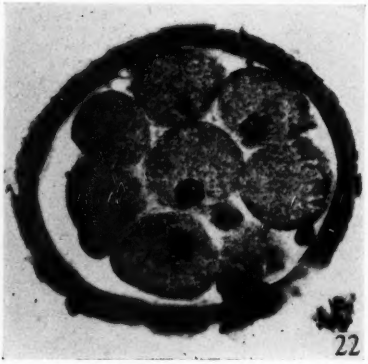

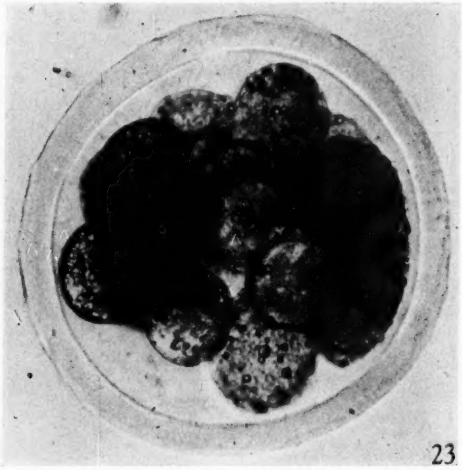

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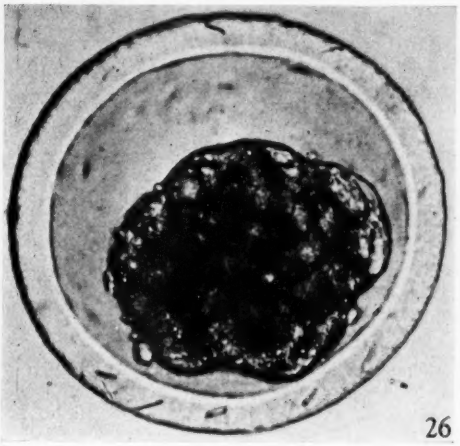

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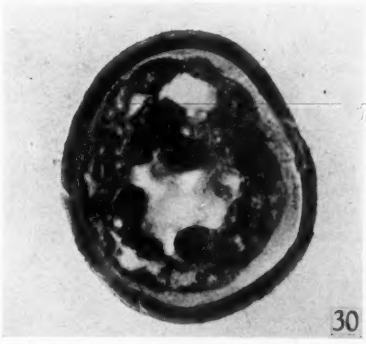

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UNIV. OF MICH.

A NEW SILVER METHOD FOR STAINING PARAFFIN SECTIONS OF THE NERVOUS SYSTEM

By G. J. ROMANES (*Beit Memorial Research Fellow*), *Anatomy School, Cambridge*

In this laboratory only Bayer's pre-war Protargol has proved satisfactory for Bodian's (1936) method for staining paraffin sections of the nervous system. In view of the difficulty of obtaining this substance an alternative method was sought, making use of colloidal silver solutions. It was considered essential that the solutions used should be easily prepared, cheap, as standard as possible and uniformly effective in the hands of technicians and research workers alike.

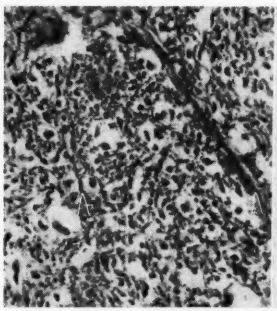

Fig. 1.

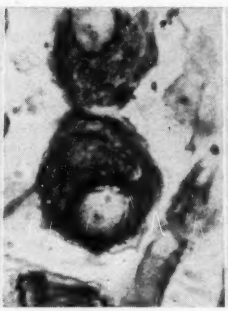

Fig. 2.

Fig. 3.

Fig. 1. Section of the lateral column from the spinal cord of a foetal sheep. Fixative, 10% formalin. 4 hr. impregnation. ×312.

Fig. 2. Section of a posterior root ganglion cell from a mouse. Fixative, 10% formalin. 15 hr. impregnation. ×650.

Fig. 3. Section of the corpus striatum from a dog. Fixative, 10% chloral hydrate in 10% formalin. 15 hr. impregnation. ×312.

The solutions are prepared as follows:

1 g. of silver nitrate (old or brown crystals are useless) is dissolved in 20 c.c. of distilled water and just sufficient dilute ammonia is added to dissolve the precipitate formed by the first few drops. This is diluted to 1 l. with distilled water. It forms a stock solution which keeps well and from which the impregnating fluid is prepared. It is important to use dilute ammonia to avoid adding excess.

The impregnating fluid is prepared by adding to 30 c.c. of the above solution 10 c.c. of 1% gelatin, slightly warmed to keep it fluid, 0·8 c.c. of 0·5% tannic acid in distilled water and 0·3 c.c. of pure pyridine, stirring the solution all the time. This solution, which gradually becomes dark brown owing to the formation of colloidal silver, should be made up shortly before use and discarded after impregnation.

METHOD

(1) Paraffin sections of formalin (neutral) fixed nervous tissue, embedded after passing through benzene, are affixed to slides with albumen. It is best to float the sections on alcohol (70%), blot with cigarette paper and dry on a hot plate to ensure satisfactory flattening and firm adherence of the sections to the slides. The sections are brought to water, which need not be distilled, and placed in the impregnating fluid for 4–24 hr. at 58° C.

(2) Rinse rapidly in water.

(3) Develop for 5 min. in:

Pyrogallol	1 g.
Hydroquinone	1 g.
Sodium sulphite cryst.	10 g.
Distilled water	100 c.c.

This developer keeps well in a stoppered bottle and can be used many times.

(4) Wash well in water. Stages 1–4 may be repeated if a more intense impregnation is required.

(5) Transfer to 0·3 % gold chloride in 2 % glacial acetic acid for 5 min.

(6) Rinse in distilled water.

(7) Leave for 10–15 min. in 2 % oxalic acid in 1 % formalin.

(8) Wash well.

(9) Treat with 5 % hypo 5 min.

(10) Wash, dehydrate, clear and cover.

When removed from the staining solution, properly impregnated sections are pale brown in colour and should not darken appreciably in the developer. Under-impregnation leads to intense staining of the background, especially in recently fixed tissue, while over-impregnation produces poor cell staining.

After correct impregnation the sections show purplish-black fibres on a clear background with reddish cells containing well marked neurofibrils. The exact time of impregnation cannot be stated but, in general, telencephalic and diencephalic tissues need longer than the medulla and spinal cord; freshly fixed material requires a more protracted impregnation than that which has been fixed for some time. For the former 10–15 hr. is usually necessary, but the latter may require only 4 hr.

This stain gives poor results with cerebral cortex unless 10 % chloral hydrate is added to the 4 % formaldehyde used for fixation. It also fails to stain boutons in the majority of cases and Purkinje cells of the cerebellum are poorly impregnated, though basket cells and climbing fibres are well stained.

Alcoholic or strongly acid fixatives give poor results, as much neuroglial tissue is stained. With formalin fixation it is not essential for the tissues to be very fresh, and good results have been obtained with tissues fixed 3 days after death. The stain should therefore be useful in studying post-mortem material and works well with rodent, carnivore, ungulate and human tissue.

The mechanism of the stain is not known, but it seems that colloidal silver alone is not active, for no staining results when sufficient tannic acid is added to convert all the ammoniacal silver nitrate to colloidal silver. This may be due to an increase in the size of the colloidal silver particles or may indicate that a trace of ammoniacal silver nitrate is essential. The latter view receives support from the fact that the addition of an ammonium salt to silver proteinates which had previously failed to stain led to some staining of the nerve fibres.

I am indebted to Mr F. Smith of the Chemistry Laboratory, Cambridge, for suggestions and help in preparing the original colloidal silver solutions.

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A TECHNIQUE FOR THE PRESERVATION OF ANIMALS FOR THE STUDY OF JOINT MOVEMENT AND FOR ROUTINE DISSECTION

By C. W. OTTAWAY,* *Zoological Laboratory, Cambridge*

Whereas a complete knowledge of joint movement can be obtained only by the study of both the living and dead subject and by paying due regard to the principles of mechanics involved, much information can be obtained by the manipulation and dissection of an animal in which freedom of articular movement has been retained.

A technique for the preparation of such a specimen, using the dog as test object, is as follows. The animal is killed by the intravenous injection of nembutal. The external jugular vein and carotid artery are exposed and cannulated, and the vessels perfused, via the jugular, with mammalian Ringer solution at blood heat. The following solution is then introduced into the jugular vein by gravity at low pressure and allowed to run in until evidence of the solution is found in the cannula in the carotid.

Glycerine	1000 c.c.
Ethyl alcohol 95 %	500 c.c.
Carbolic acid	75 c.c.
Sat. soln. arsenious acid	10 c.c.

The amount of solution required is of the order of 1 l./10 kgm. body-weight. The injection should be complete within ½ hr. The vessels are then ligated, the cannulae removed and the skin incision closed with sutures.

At first, some degree of rigidity is observed in the muscles; this is most marked in those muscles of the limb which have long tendons of insertion. This rigidity, however, soon wears off, especially if the body is massaged and the joints are freely moved. Viscera are always the most difficult of tissues to preserve and if possible the animal should be starved beforehand and kept in a cool place for a day or two after injection.

This technique can also be employed on an isolated limb. A suitable artery and vein are freed from surrounding tissues and cannulated for perfusion and the injection of the fluid; the fluids are injected into the artery instead of the vein. If the limb has been removed from the animal for some hours, it is better to dissect while fresh and immerse the specimen in the fixative for 6 weeks to allow of thorough impregnation. If it is not possible to complete the dissection before preservation is necessary, the limb should be stored in the solution during the course of dissection. The dissected specimen, whether it be part of an injected animal or an immersion preparation, can be allowed to dry without affecting its state of mobility although immersion in the fluid for a few days may be required occasionally. Specimens of equine joints, dissected by the writer while fresh and immersed for an initial period of 6 weeks and then allowed to dry, are still freely moveable after a period of 6 years.

If, after fixation, dissection is delayed for upwards of 3 weeks to 1 month, the hair and stratum corneum tend to peel off and the skin to wrinkle and harden a little. This, however, has no effect on the state of preservation of tissues in general. Dissection of a dog 6 months after injection was still fruitful and there appeared no reason why dissection could not have been further delayed.

This technique has been used to obtain an appreciation of muscle and joint movement both by X-ray (including screening and cinematography) and by dissection. It is not suggested that final conclusions can be determined by this method, but the results obtained are very helpful.

Apart from its value for the study of joint movement, the technique is of great value in routine dissection. The vessels are not conspicuous as the injecting fluid is colourless; but as their lumina remain patent and a state of elasticity persists, there would appear to be no reason why they should not be injected with colouring matter after preservation. No attempt has been made so to inject a whole animal, but an 8 cm. length of artery from a dog which had been dead for 6 months was injected satisfactorily with a solution of plaster of Paris and safranin.

The muscles retain their natural colour, are freely separable one from another and retain a high degree of stretch so that an appreciation of their action can be obtained. The nerves stand out white among the red muscle fibres. The fat is set but remains pliable. Fascia is translucent and can be differentiated easily from the smallest tendon fibres, which preserve a glistening appearance, while the 'lubrication' of the fasciae facilitates their separation from adjoining tissues in a manner similar to that experienced in dissection of a recently killed unfixed animal.

* Wellcome Research Fellow of the Veterinary Educational Trust.

Another interesting point is the ease with which serous membranes can be traced. The peritoneum and pleura strip freely from the body wall and the two layers of the mesentery can be separated one from the other. The laryngeal and tracheal cartilages are freely movable. The organs retain their natural colour and can be moulded into shape as required. The brain and spinal cord although fixed do not show to particular advantage for ordinary dissection purposes as, owing to the absence of formalin, the fixative is essentially not a hardening agent.

The value of this fixative for histological work is at present being investigated.

SUMMARY

A technique is described for the preservation of animals by the intravenous injection of a glycerine-alcohol-carbolic-arsenic solution. The tissues remain pliable and retain their natural colour. The specimens can be used for an appreciation of joint movement and for routine dissection.

I wish to thank Professor J. Gray and Dr W. R. Wooldridge for their encouragement in the preparation of these specimens.

ANATOMICAL NOTES

PATENT FORAMEN OVALE AND DUCTUS ARTERIOSUS IN A LION CUB

By K. J. FRANKLIN, *Nuffield Institute for Medical Research, Oxford*

The lion cub forming the subject of this note was born in the Menagerie of the Zoological Society of London on 26 February 1946, and died on 5 March 1946. No clinical history is available because the mother was very savage and aggressive, and the keeper-in-charge could not see the cub closely, but only at some distance in the dim light of the inner den in which it was born. He did not, under this disadvantage, notice any indication of illness in the cub before it was found dead.

Post mortem the eyelids were closed (in lion cubs they do not open until the 6th to the 10th day post partum), and the lungs were inflated and *appeared* to be congested (? on account of post mortem changes).

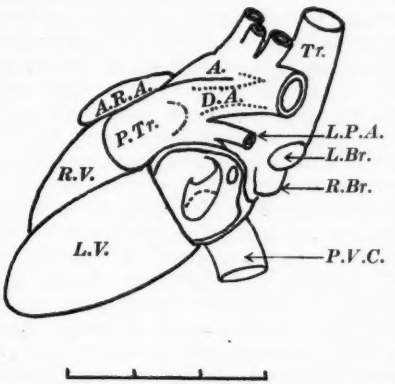

Fig. 1. Lion (*Felis leo*) cub, 7 days post partum. Outline drawing of heart, etc., viewed from the left side, with the auricle of the left atrium removed to show the valve of the foramen ovale and, just dorsal to it, the common opening of the pulmonary veins from the apical and cardiac lobes of the right lung. *P.V.C.*, posterior vena cava. *A.R.A.*, auricle of right atrium. *R.V.*, right ventricle. *P.Tr.*, pulmonary trunk. *L.P.A.*, left pulmonary artery (a small portion of the right artery is just visible between it and the wall of the left atrium). *D.A.*, ductus arteriosus (internal calibre and junction with aorta indicated by dotted lines). *L.V.*, left ventricle. *A.*, arch of aorta. *Tr.*, trachea. *L.Br.*, cut left bronchus. *R.Br.*, cut right bronchus. Dash-dot line indicates origin of common pulmonary artery. Interrupted line indicates cranial limit of crista dividens (free caudal edge of inter-atrial septum). Scale in cm.

The heart and great vessels were examined after they had been fixed in 5% formol saline; their dissection revealed the following:

The apposable portion of the via sinistra (the valve of the foramen ovale) was of the long flap type usual in *Felis leo*, with the free border ending in two cornua (the dorsal one extending considerably farther cranially than the sternal one) and the centre of this free border lying well cranial to the crista dividens (free caudal edge of the inter-atrial septum). Immediately dorsal to the valve was the common opening of the veins draining the apical and cardiac lobes of the right lung. There was some blood in the via sinistra (foramen ovale), i.e. it was presumably patent up to death.

The calibre of the pulmonary trunk considerably exceeded that of the beginning of the aorta, as is common in the foetus towards full term. The trunk, after giving off the common pulmonary artery, was continued (functionally) by a fairly wide ductus arteriosus about 12 mm. long and somewhat narrower internally at the middle of its course than at either end. The junction of the ductus with the much narrower terminal portion of the arch of the aorta exhibited no valvular arrangement, but merely the curved edge of union that one would expect from the meeting of two tubes at the angle shown in the figure. A ductus arteriosus which has contracted during or shortly after birth may relax if the newborn animal is very short-lived. But it seems unlikely that the ductus in this cub, which lived for a week, was contracted at the time of death and thereafter relaxed. It may, on the other hand, have contracted in the normal way at birth and thereafter relaxed, for this happens if the newborn animal's condition deteriorates, and the mother of this cub is notorious for her poor treatment of her offspring (usually she either deserts or eats her progeny).

It would seem reasonable, therefore, to ascribe the death of the cub to patency of the two foetal cardiovascular by-passes, continued unduly after birth.

The writer wishes to thank Colonel A. E. Hamerton, C.M.G., D.S.O., for the specimen and for information about the cub and its mother. The relevant literature is noted below.

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THE LAXATOR TYMPANI MUSCLE

By E. W. WALLS, *University College, Cardiff*

Recently during routine dissection of the petrous temporal bone, three specimens have been met with in which muscle could clearly be seen attached to the anterior process of the malleus. Reference to the more commonly used text-books did not prove helpful, and it was only when earlier authorities were consulted that the muscle was identified as the laxator tympani.

Soemmerring (1806) in his *Icones Organi Auditus Humani* (Table 2, Fig. 10) shows four muscles related to the tympanum. These are: (1) the laxator membranae tympani, (2) the m. externus mallei, (3) the tensor tympani, and (4) the stapedius. The first is sometimes referred to as the laxator tympani minor of Soemmerring, but is in fact not muscular but ligamentous, connecting the handle and short process of the malleus to the tympanic membrane. The second muscle, the m. externus mallei (sometimes called the laxator major of Soemmerring) is the true laxator and has the following parts: an inferior tendon attached to the spine of the sphenoid, an intermediate fleshy rhomboidal part, and a superior tendon which passes through the petro-tympanic fissure to join the long process of the malleus.

Cloquet (1825) copied from Soemmerring, without acknowledgement, the figure referred to above. In it the m. externus mallei was renamed, and appeared as 'le muscle antérieur du marteau'; it is interesting to note that in certain of the anatomical models made on the continent and frequently used for teaching in this country, 'le muscle antérieur du marteau' is prominently labelled.

The first edition of Gray's well-known text-book (1858) described two laxator muscles, (1) the laxator tympani major of Soemmerring, supplied by the chorda tympani nerve; (2) the laxator tympani minor of Soemmerring. Gray stated that some anatomists regarded the second muscle as ligamentous, an opinion which is now accepted. With regard to the laxator tympani major, Schäfer (1882), in describing the anterior ligament of the malleus, wrote: 'the part of the ligament which passes out of the Glaserian fissure was long thought to be muscular (laxator tympani auct.), but most observers agree in denying the existence of muscular tissue in this situation,' but Holden & Langton (1868), Heitzmann (1887), Macalister (1889), Gegenbaur (1896) and Morris (1898) all acknowledged the occurrence of the laxator tympani, their descriptions varying with regard to its frequency and degree of development. Holden agreed with Gray in giving its nerve supply as coming from the facial nerve, but as the muscle arises from the spine of the sphenoid and reaches the long process by passing through the petro-tympanic fissure it seems hardly likely that the nerve of the second arch would supply it.

Histologically the tissue presents the characters of voluntary muscle so far as fibre arrangement and nuclear size and position are concerned, but not as regards cross-striation. Only an occasional fibre shows striation and that very faintly indeed. Possibly with quite fresh material and immediate fixation this striation would be better marked; recent work, however, by Perlman & Case (1939) appears to show that the activity of the muscles of the tympanic cavity is purely reflex, constituting, as Mainland (1945) puts it, 'an accommodation mechanism somewhat as in the eye'. No doubt, when present, the laxator acts in the same way, so that a structure identical with voluntary muscle is hardly to be expected.

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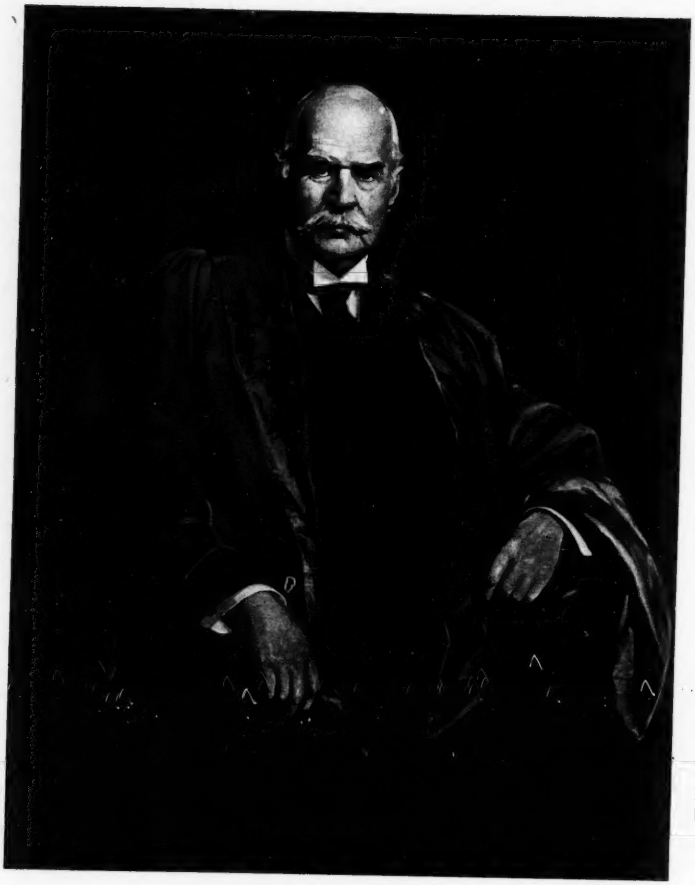

THOMAS HASTIE BRYCE

1862–1946

UNIV. OF MICH.

IN MEMORIAM

EMERITUS PROFESSOR THOMAS HASTIE BRYCE, M.A., M.D., LL.D., F.R.S.

Dr T. H. Bryce, Emeritus Professor of Anatomy in the University of Glasgow, died on 16 May 1946 at Oxford. He had gone there from his own home at Peebles in the year before to be with his son; he was in his 84th year.

Thomas Hastie Bryce was born in Edinburgh on 20 October 1862. He was the son of William Bryce, M.D., a physician practising there, whose forbears were Ulster Scots distinguished enough still to be remembered in Ulster. He had as cousins, all a little senior to him but with whom he was intimate, Robert Young, architect in Belfast, who was among those at the beginning of field archaeology in Ulster, Viscount Bryce, Regius Professor of Civil Law at Oxford and sometime Ambassador at Washington, and John Annan Bryce, scholar of Glasgow and Oxford and a distinguished man of affairs. He shared to the full the common heritage of these men, the sense of the need of law and order in life, of the importance of the past in its development, and of the dignity that can come of the recognition of one's own place in it.

Bryce had his early education at Edinburgh Collegiate School and afterwards took the Arts and Medical courses at the University of Edinburgh, graduating in Medicine in 1886. After further hospital training and some experience of general practice he was for a short time a demonstrator with Sir William Turner. Still undecided as to his future, he moved to Glasgow in 1888. The west always had an attraction for him; 'it's so much older, and it's more comfortable' he used to say; and so he settled down to private practice in Glasgow, and almost at once began his studies of its Cathedral and its records.

In 1889 he married Mary, daughter of George Wilson of Dalmarnoch, a lady of winning personality and great gifts, an acknowledged musician, an accomplished artist, and a notable embroiderer; and there is no doubt that much of his further development was defined for him through her. She died in 1934.

In 1890 Bryce was appointed to the Lectureship in Anatomy at Queen Margaret College for Women. In 1901 he was awarded the M.D. with a Gold Medal. When, in 1892, the Lectureship at Queen Margaret College for Women was made a University Lectureship he was confirmed in that appointment. So began his long association with the University of Glasgow and the forty-five years of devoted service that he gave to it. From the beginning he gave to the University his full allegiance, and his pride in its activities increased as he took an ever greater part in its government, till at the end it could be said of him that he was one of its wisest and most devoted senators. He liked to remember that his grandfather, the Rev. James Bryce, had matriculated there in 1781.

He remained at Queen Margaret College till 1909 when he was appointed to the Regius Chair of Anatomy in the University on Professor Cleland's resignation. He held the Chair till 1935, the year after the death of his wife; and on his retirement then, in his 73rd year, he was made Emeritus Professor and received the Degree of LL.D.

He was elected to the Royal Society in 1922.

He had been President of the Anatomical Society from 1930 to 1932.

Professor Bryce will be remembered by those who knew him for his open genial greeting, the ease of his friendliness and the kindly equality of his way with a junior; for he was warm in human fellowship and he sought it freely everywhere. His charm of manner and old-world courtesy and his genuine enjoyment of humour were all very pleasant things. He was essentially a countryman; an active golfer, a keen fisherman, a diligent gardener; to know him one must needs have been with him in the country and especially at his beautiful home at Peebles. There one learnt that his abiding interests were in the countryside and its ways, its lore, its people, and its history; that he lived in these things and that they lived in him; that against the background of them lay his interest in the Humanities and his wide knowledge of literature, history—especially Scottish history—and affairs; and that it was from them that there grew up in him the need for quality in men and things and a love of beauty in all its forms. He has left records of this side of his personality in the department he designed for Queen Margaret College, in the staff quarters he brought into being in the University, and in his curatorship of the Hunterian art collections. He himself chose James Gunn to paint the portrait of him presented to the University in 1931 as the gift of his former pupils and friends, and which accompanies this note.

His background of general culture well suited him for the old status of Professor in Scotland; he had

retained command of his higher mind and was not likely to lose it in the factual details or elaborate techniques of a professional subject. It has thus been well said of him that he was the last distinguished figure of the old school of anatomists; those with the background of the something more, with the broad general outlook on structure as a part of a whole and with the quick recognition of the essential fact. The volumes on Bones and Joints (1915) and Muscles (1923) in the 11th edition of Quain's *Anatomy*, of which he was editor, attest these qualities of his mind; nowhere are there more complete or better balanced summaries of the knowledge of the time.

He must have trained himself in Archaeology and Physical Anthropology, for these subjects were not much considered in Cleland's school; but he soon became known among the field workers in Scotland. He was appointed a member of the Royal Commission on Ancient Monuments in 1908, and he was always afterwards active on its behalf, travelling, inspecting, and reporting; and much of his labour is stored in its records. His best work was done in Arran. His descriptions of the megalithic monuments there, his dating of them, the cultural sequences he established and their relation to the sequences in Ireland are all complete and exact work; it is typical that he speculated much on the meanings of the change from inhumation to incineration burial and the lateness of its happening in Arran. He collected his observations in *The Book of Arran* (1910). His knowledge of the physical structure of the people of Scotland was based on the extensive examinations he made of their remains, for most of the material that was found in Scotland came in the end to him. He was latterly assisted in this work by Matthew Young, his first Senior Demonstrator, that shy genius whose early death affected him much, and who had already written *The Craniology of the Scottish People*, a work which honoured his chief and established his own name. Professor Bryce stated his own position and gave his own conclusions in *The Anthropological History of the Scottish People* which he took as the subject of the Rhind Lectures at the University of Edinburgh in 1924.

It was in the subject of Development that he did his most exact work, and thereby established his contention that breadth of endeavour need not mean the loss of the powers of precision. He must have begun his studies in Embryology soon after his appointment to Queen Margaret College, drawn to the subject no doubt as a then relatively young science and as a study in the logic of causation. He worked first on the blood of Lepidosiren, and then on the development of the spleen, the histogenesis of nerves, the development of the spinal roots, and much else besides; and from 1900 onwards he was publishing his results which as early as 1906 gained for him the Keith Prize of the Royal Society of Edinburgh, of which he had already become a Fellow in 1898. It was in the early stages of human development and the mechanism of uterine implantation that he was most greatly interested, stimulated no doubt by Teacher's discovery and by the need to interpret what had not been interpreted before and to explain that about which so little was known.

The results of the work on the Teacher-Bryce ovum (T.B.1), an ovum which must always have its place in the literature and history of Human Embryology, were published in 1908 (*The Early Development and Imbedding of the Human Ovum*). It is strange to remember now that criticisms of weight were made against his interpretation, but he held to his descriptions and they have been found correct: and when he himself reviewed the work 20 and 25 years later in his Royal Society and Royal Society of Edinburgh papers there was nothing to alter and only a little to add. The volume of Quain's *Anatomy* on Embryology also appeared in 1908.

'Tommy Bryce' was to medical students one of the great figures of the Glasgow School. His was not a style to appeal to the ordinary student, however, and it was in the small science classes or in the meetings with his staff or in visits to the museums that he really shone. These were occasions never to be forgotten. There was the cautious opening of the discussion, the matter to be talked of defining itself, the growth of it as his mind warmed to its work, and then the rapid sustained enthusiastic outpouring of fact woven into a pattern that had the unity of a whole. This was not the mechanics of instruction; this was the discipline of education in affairs of the mind. One appreciated there the width of his knowledge, the sense of proportion, the generosity of his views on the work of others; but more than all one felt the quality in his talk. It is still true to say that the quality is the man; there are many who remember that teaching with gratitude and the man with affection.

T.W.

Anatomical Society of Great Britain and Ireland

PROCEEDINGS

May 1943 — *November* 1944

June 1945 — *November* 1945

February 1946 — *June* 1946

RECORDED BY

R. WHEELER HAINES

PROCEEDINGS OF THE ANATOMICAL SOCIETY

MAY 1943

The Annual General Meeting of the Society for the Session 1942–3 was held in the Anatomy Department of the Middlesex Hospital Medical School, Mortimer Street, W. 1, at 10 a.m. on Friday and Saturday, 21 and 22 May 1943, the President (Dr W. H. L. Duckworth) in the Chair.

Professor Le Gros Clark presented a paper by Dr C. Singer entitled 'To Vesalius on the fourth centenary of his *De Humani Corporis Fabrica*, June 1543'.

This paper is published *in extenso* in Vol. 77, pp. 261–5 of the *Journal of Anatomy*, 1943.

Dr G. W. Harris read a paper on 'Some effects of remote-control stimulation of the pituitary gland', of which the following is an abstract:

The ultimate aim of recent experiments was the elucidation of the nerve supply of the pituitary gland and of the possible control exerted over the gland by the hypothalamus.

The technique employed was remote-control stimulation of the hypothalamo-hypophysial mechanism, as previously demonstrated to the Society (Harris, G. W., 1942, *J. Anat., Lond.*, **76**, 226).

So far it has been found that stimulation of the gland inhibits water diuresis, increases relative chloride excretion and has little effect on blood sugar.

Professor W. J. Hamilton read a paper on 'Stages of maturation and fertilization of the human ovum'.

An unsegmented human ovum at the stage of the 2nd maturation spindle was recovered from the uterine tube on the 17th day of the cycle. Its histological structure is described. A second ovum showing sperms in the zona pellucida is also described.

This paper is published *in extenso* in Vol. **78**, pp. 1–4 of the *Journal of Anatomy*, 1944.

Professor W. J. Hamilton read a paper on 'A previllous human embryo', of which the following is an abstract:

The embryo here described was found in a uterus removed by hysterectomy on the 25th day of a uterine cycle of 28 days' duration. In the unfixed state it appeared as a slightly raised translucent area on the posterior wall of the uterine body, clearly demarcated from the surrounding endometrium.

After fixation the area became opaque, but was still raised above the level of the surrounding endometrium. The block of endometrial tissue containing the embryo was cut in a faultless series of sections (7μ) by Mr E. Park. The trophoblastic portion of the chorionic vesicle was present in 133 sections. The calculated dimensions of the vesicle were approximately $0{\cdot}931 \times 0{\cdot}770 \times 0{\cdot}727$ mm.

The trophoblast is differentiated into a cytotrophoblast and a syncytiotrophoblast. The trophoblastic shell varies in thickness, being thinnest in its abembryonic region, over part of which the uterine epithelium is deficient. The peripheral edge of the trophoblast is clearly demarcated from the endometrial tissue. A small amount of blood is found in the intercommunicating lacunae of the syncytiotrophoblast.

The embryonic disk ($133\mu \times 118\mu$) is present in 19 sections. The amniotic cavity is present and the yolk-sac is in process of formation.

Professor A. J. E. Cave read for Professor E. J. R. Evatt a paper on 'Unusual ossification of the manubrium sterni', of which the following is an abstract:

In two of four sterna obtained from hunchbacked children, the manubrium sterni was bipartite, being divided by a transverse intramanubrial joint of primary cartilaginous nature into a larger superior and a smaller inferior segment. Details of the gross anatomy of these two specimens are submitted. The occurrence of two ossificatory centres (a cranial and a caudal) in the developing manubrium is not unknown: no previous record, however, appears available concerning the formation of a bipartite manubrium from failure of the embryonic coalescence of such centres.

Professor T. Rogalski read a paper on 'Crossed and uncrossed optic fibres in microphthalmia', of which the following is an abstract:

An 11-weeks-old child shows left hare-lip and congenital absence of the left eyeball. The left corneo-scleral region is represented by a ridge containing melanine pigment, showing, in serial sections, traces of coloboma ad limbum with atrophied orbito-palpebral cyst containing capillaries, ciliary muscles, nerves and melanine cells. These last are chromatophores of neuroblastic origin. The left retina, optic nerve, lens and iris are completely wanting, but the remaining orbital contents are

present. The chiasma is represented by the right optic nerve alone; the right optic tract is but two-thirds the bulk of the left. The right lateral geniculate body is slightly smaller than, and differs in shape from, the left. Following Le Gros Clark's notation, the cellular stratification is: left = 1, 0, 3, 0, (5+6), right = 0, 2, (3+4+5), 6, the bracketed figures indicating parts occupied by scattered cell-clumps or cells instead of by definite cell-layers. The cells corresponding to uncrossed fibres occupy much less space than those corresponding to crossed fibres; the same is true of tractus fibres entering the nucleus and of emergent geniculo-cortical fibres.

The right occipital lobe is the shorter. Serial sections demonstrate differences of topographical arrangement of the striatal areas of the two sides. At the lips and bottom of the postcalcarine sulcus occur areas lacking striatal pattern, but differently located on the respective hemispheres. Arrangements present suggest the occurrence of three areas on either side, viz: (1) the largest (5·65 sq.cm.), associated with the endings of crossed and uncrossed fibres; (2) a smaller (2·54 sq.cm.), for crossed fibres only; (3) a smallest (1·58 sq.cm.), for uncrossed fibres. Present findings do not support the general notion of a single cortical area for both crossed and uncrossed fibres; they are being checked by study of the normal neonatal visual cortex.

Professor C. F. V. Smout read a paper on 'A pseudo-hermaphrodite', of which the following is an abstract:

The viscera of a pseudo-hermaphroditic child aged 7 years are exhibited and described. The external genitalia are of the male type; the pelvic organs are of female type, though a prostate gland is present. The bony pelvis conforms to the female pattern. Its ossification appears to have been selective, certain portions of it having attained a degree of development usually associated with a pubertal or post-pubertal age, other portions remaining rudimentary.

Professor A. B. Appleton read a paper on 'Bronchi and pulmonary vessels in the upper lobe of the right lung'.

The arrangement of broncho-pulmonary segments has been examined with reference to the openings into the upper lobe ('eparterial') bronchus. Various arrangements have been found associated with each pattern of the openings.

The pulmonary arteries follow the smaller bronchi and bronchioles. Those of the upper lobe are derived from the lower, as well as from the upper branch of the right, pulmonary artery. Very variable portions of the pectoral and posterior segments of this lobe are provided with arteries from the lower pulmonary branch.

Variations in the pattern of venous drainage are described. Differences in the manner of union of the larger arteries and veins close to the hilum affect the appearances in this region, especially from the lateral aspect as viewed by opening up the fissures.

This paper is published *in extenso* in Vol. 79, pp. 97–120 of the *Journal of Anatomy*, 1945.

Drs G. Weddell and M. A. Falconer presented a paper on 'Asymmetry of the thoracic inlet in relation to the scalenus anticus syndrome', of which the following is an abstract:

A number of Service personnel complain of symptoms referable to vascular disturbance in the upper extremities following unaccustomed strain on the shoulder girdle. Three cases are described in which there was direct and indirect anatomical evidence that the symptoms were due to a compression of the subclavian vessels between the first rib and the clavicle. Evidence is also brought forward that forced movements of the shoulder girdle in many normal persons cause transient vascular disturbances in the arm. Methods of differentiating vascular disturbances due to costo-clavicular compression and the scalenus anticus syndrome are discussed.

Drs E. C. Amoroso and J. H. Jackson presented a paper by themselves and the late Dr W. F. B. Griffiths on 'The development of the cat during the first fourteen days', of which the following is an abstract:

Recent accounts of the early development of mammalian eggs led to re-study of *Felis* material. The observations submitted, which partly confirm the findings of Hill and Tribe, are based on photomicrographs of the living egg and on serial sections of ova of known post-coital age.

Particular attention is called to the duration of the tubal journey and to the changes undergone by the egg during its tubal sojourn. The precise location of the egg at the time of inception of the blastocyst cavity is shown. Details of the course of development of the blastocyst up to and including the time of its first attachment are given. Reference is made to the artificial insemination of the cat.

Professors W. J. Hamilton and J. D. Boyd with Dr D. M. Samuel presented a paper on 'Observations on blastocysts and chorionic vesicles of the sheep'.

Blastocysts and chorionic vesicles of known age were obtained between the 7th and 20th days after insemination. Photographs of the living and of the fixed blastocysts, and representative sections thereof, are demonstrated and discussed.

Professors J. D. Boyd and W. J. Hamilton with Dr J. Hammond, Jr., presented a paper on 'Trans-uterine migration of sheep ovum'.

Data are presented on the occurrence of trans-uterine migration of the ovum in the sheep and other mammals.

This paper is published *in extenso* in Vol. 78, pp. 5–14 of the *Journal of Anatomy*, 1944.

Dr F. P. Reagan read a paper on 'Remnants of left eruncus transversus in injected human foetuses', of which the following is an abstract:

Since Marshall's great work, little attention has been paid to the degenerative phases in the development of the left truncus transversus (duct of Cuvier) in man. Kampmeier (*Morph. Jb.*, 1931) made incidental reference thereto in his studies on human lymphatics; his figures are valuable in determining the stages at which this degeneration begins: some of them show a left Cuvieran duct unaccountably near the jugulo-subclavian junction.

Senior (in Morris's *Anatomy*, 1933) describes and figures a 'ligament of the left vena cava superior', but it is not clear from his figures how the 'ligament' could connect with the oblique vein of the left atrium. Little attempt is made in current British text-books to illustrate this structure, and the accounts and figures given therein are difficult to harmonize. Such accounts show the ligament or fold as attached to the posterior aspect of the left pulmonary artery. In none is it readily appreciable how a vessel draining the fold could course superiorly to enter the vena triradica (=left superior intercostal vein: superior hemiazygos vein of various authorities) whilst maintaining a morphological relationship comparable to that borne by the superior vena cava to the right pulmonary artery and the transverse pericardial sinus.

The conditions disclosed by special examination of injected human foetuses are therefore described and their significance is discussed.

Professor S. Zuckerman and Dr S. H. Green presented a paper on 'Factors in ovarian growth', of which the following is an abstract:

The development of primordial ova and of the follicle in its early stages does not appear to be under the control of the pituitary gland. Both oestrogens and androgens depress the gonadotrophic function of the pituitary, and in this way inhibit follicular development in its later stages. Follicular hormone, however, also appears to have a direct effect in destroying ova and follicles in their earlier phases of growth, whereas androgens stimulate at this stage.

Dr P. Glees read a paper on 'Observations on the neurohistology and fibre connexions of the neostriatum'.

A study has been made of the neo-striatum of the rabbit, cat and monkey. With the help of the Golgi method the various cell types and their distribution have been demonstrated. By means of a special silver technique the pericellular networks are shown. This plexus includes myelinated fibres (as seen in Weigert preparations) which collect in characteristic bundles before they penetrate the internal capsule to form the striofugal system. After lesions have been made in the caudate nucleus of the rabbit these fibre bundles can be traced in Marchi section to the hypothalamic region. This connexion demonstrates that the caudate nucleus not only relays in the pallidum but has its own extrinsic connexions. Following cortical lesions in the cat a terminal degeneration of cortical collaterals in the caudate nucleus can be observed, thus supporting the physiological evidence that the suppressor areas of the cortex are connected with the caudate nucleus. This connexion is myelinated only on its way through the internal capsule and becomes non-myelinated as soon as the collaterals turn medially towards the caudate nucleus. The myelinated cortico-striate connexions described by some previous investigators are believed to have no real existence.

This paper is published *in extenso* in Vol. 78, pp. 47–51 of the *Journal of Anatomy*, 1944.

Mr S. Wilson Charles read a paper on 'The development of the paranasal sinuses of the pig', of which the following is an abstract:

The development of the paranasal sinuses and associated bones in the pig is described from the date of commencement of ossification up to the age of 6 months.

It is commonly stated that the growth of the air sinuses is due to excavation or absorption of certain skull bones, but this is not correct for the true paranasal sinuses. These sinuses are differentiated as diverticula of the middle meatus before ossification begins, and are separated by the paranasal cartilages from the bones with which they become subsequently associated. These cartilages are gradually de-differentiated or absorbed until the bones and sinuses come into direct contact. The sinuses are enclosed primarily by the growth of the bones, though from the age of 5 or 6 months onwards they may invade, or become associated with, the cavities which are being formed in the diplöe of adjacent bones.

The examination of the bones associated with the paranasal sinuses is of interest because it demonstrates that these bones do not move or expand by a process of reformation in the manner described in the madder interpretations.

Dr R. Wheeler Haines read a paper on 'The mechanism of opposition and reposition of the thumb'.

The rotation of the pollical metacarpal on the trapezium that occurs as the movements in opposition near completion is determined by a strong posterior oblique ligament. This ligament runs from the posterior surface of the trapezium to a small projection on the medial aspect of the base of the metacarpal, and becomes taut as the carpo-metacarpal joint is flexed. A similar but weaker anterior oblique ligament, running from the anterior surface of the trapezium to the same region of the metacarpal, determines the rotation in the opposite sense that is associated with reposition.

This paper is published *in extenso* in Vol. **78**, pp. 44–6 of the *Journal of Anatomy*, 1944.

Dr J. J. Pritchard read a paper on 'Principles of growth and repair in membrane bones', of which the following is an abstract:

From an analysis of membrane bone growth in rats, rabbits, mice and man the following general principles emerge.

Osteogenic cells first appear within masses or sheets of condensed mesenchyme. They proliferate by mitotic division in a centrifugal direction, the more centrally placed cells becoming orientated in single layers around invading blood vessels, and laying down a perivascular bony lattice, lined everywhere by a single layer of highly polarized osteogenic cells.

The peripheral mesenchyme differentiates fibroblasts which lay down collagen fibres; the endosteal perivascular mesenchyme becomes loose and remains free of fibroblasts and collagen.

The peripheral masses of osteogenic cells invade the sheets of condensed mesenchyme and lay down a two-dimensional bony lattice with similar relations to blood vessels, and differentiating fibroblasts as described for the three-dimensional lattice.

Suture formation usually occurs within these sheets. Its location is at first undetermined by any differentiation of cells or matrix. Advancing sheets of proliferating osteogenic cells are insulated from each other by fibroblastic envelopes. Their meeting results in the formation of a double fibroblastic membrane separating adjacent bone territories, which becomes the definitive sutural membrane as collagen fibres appear within it. The osteogenic cells make no attempt to traverse this fibrous barrier.

This early perivascular bone becomes laminated and increases in thickness and compactness as the lining osteogenic cells continue to lay down matrix. Marrow spaces are produced by absorption of the perivascular bone lattice with production of numerous osteoclasts. Endosteal connective tissue thus liberated remains collagen-free.

Membrane bone regeneration shows many analogies with the foregoing.

Two kinds of 'granulation tissue' result from the stimulus of injury: (*a*) an *osteogenetic* by the subperiosteal and endosteal proliferation of osteogenic cells and blood-vessels in a fibroblast-free environment, and (*b*) a *fibroblastic*. The former recapitulates osteogenesis as described for the developmental stage, with production of a perivascular bony lattice applied to the remaining bone fragments: osteoclasts also appear around the edges of the dead bone near the fracture line. The latter differentiates collagen fibres which tend to surround and isolate the osteogenic territories.

Fibrous union is thus the rule where the osteogenetic regions are separated by more than a millimetre or two, and is analogous to the development of a sutural membrane. Bony union depends on the fusion of the separate osteogenetic granulation tissues before fibroblastic insulation has occurred. Cartilage is rarely seen during the repair of the flat membrane bones.

Dr B. Feinstein, introduced by Professor W. E. Le Gros Clark, read a paper on 'The pattern of innervation of voluntary muscle', of which the following is an abstract:

During the course of investigations connected with peripheral nerve injuries in man, a knowledge of the exact mode of innervation of voluntary muscle became essential. The problem was investigated under three headings: (i) by dissection of cadavers to note the position of the so-called 'motor points' in the limb muscles; (ii) by tracing of nerve fibres from their 'motor point' to the small nerve trunks giving rise to motor end-plates within the muscle (by dissection under a binocular microscope after clearing of muscles from experimental animals); (iii) by study of the general pattern of innervation and distribution of motor end-plates in experimental animals, after staining intra-vitally with methylene blue. The last method is also being used to study the pattern of re-innervation of muscle.

The results of these investigations have been correlated with the electrical activity of voluntary muscle following denervation until regeneration occurs.

Dr E. Gutmann and Professor J. Z. Young, introduced by Professor W. E. Le Gros Clark, presented a paper on 'The re-innervation of muscle after various periods of atrophy'.

The structure of normal motor-plates in muscle fibres is described. When a muscle fibre undergoes atrophic shrinkage its end-plate may remain intact even after more than a year of denervation. After a nerve has been interrupted by crushing close to a

muscle, one nerve fibre returns to each plate. After longer periods of atrophy a portion of the returning stream of axoplasm often 'escapes' and makes an ultra-terminal fibre; some end-plates are not re-innervated, and new plates are formed where the ultra-terminal fibres touch the sarcoplasm. When muscles have been kept denervated for increasing periods, the proportion of old end-plates which become re-innervated is progressively reduced. The effectiveness of re-innervation becomes prejudiced by at least three factors: (*a*) the increasing difficulty and slowness with which new plates are formed; (*b*) the abnormality of the pattern of re-innervation, increasing the likelihood of wrong connexions; and (*c*) the fact that many muscle fibres are never re-innervated at all.

This paper is published *in extenso* in Vol. **78**, pp. 15–43 of the *Journal of Anatomy*, 1944.

NOVEMBER 1944

The Annual General Meeting of the Society for the Session 1943–4 (postponed from November 1943) was held in the Anatomy Department of King's College, Strand, at 10 a.m. on 25 November 1944, the President (Professor F. Wood-Jones) in the Chair.

Professor A. St G. Huggett and Dr J. J. Pritchard presented a paper on 'Intrauterine death and its consequences in the rat'.

At certain stages of gestation in the rat both ovariectomy and injection of follicle-stimulating hormone result in foetal death. Spontaneous death *in utero* is also frequent. The consequences of foetal death in these different circumstances and also as the result of deliberate operative crushing were investigated, with special reference to the histological changes occurring in the surviving placentae.

In all cases studied intrauterine death led to sloughing of the decidua parietalis and re-establishment of the lumen with spreading epithelialization. The yolk-sac and labyrinthine portion of the placenta ceased to develop further and soon degenerated.

After crushing of the foetuses of one horn, their placentae underwent comparatively normal development of their giant-cell and reticularis components until the 19th day, when widespread haemorrhagic infarction occurred: the decidua basalis and metrial glands were normal.

Ovariectomy at the 10th day quickly kills foetuses: the surviving placentae begin to develop as in the crushed cases, but spreading necrosis of the decidua basalis results in placental infarction before the 14th day.

F.S.H. in a single injection of 50–100 I.U. at the 10th day causes foetal death in 2–4 days. The decidua basalis is very abnormal, being much thicker and less vascular than controls, and exhibiting centripetally spreading necrosis. The placenta shows great increase of its giant-cell zone before its destruction at the 14th to 15th day. Great hypertrophy of the uterine mucosa, which is thrown into villous folds, is a feature of these cases.

Spontaneous foetal death leads to deciduoma production in early pregnancy but to temporary survival of greatly modified foetal remnants in later cases.

There is evidence that in all except the crush cases, failure of vascularization of the yolk-sac and allantois precedes death.

The main conclusion is drawn that the placenta survives foetal death so long as decidua basalis development does not interfere with its blood supply.

Professor Nicol asked whether any changes in the glycogen content of the decidua or placenta had been noticed following the injection of follicle-stimulating hormone, for in the ferret the glycogen is increased in the follicular stage and decreased in the luteal stage of the cycle, and appears to be under the control of the follicular hormone.

Professor Boyd referred to Schneider's work on the persistence of the placenta after the removal of the embryo in the rabbit, and asked for details of the crush injury.

Dr O'Connor commented on the existence of a critical stage in development, after reaching which the placenta would continue to its predestined state in spite of removal of the foetus, and compared the placenta, in this respect, with other organs. He asked whether there was any tendency to malignancy in the placental tissues.

Professor West inquired as to the method by which the foetus was absorbed so rapidly.

In reply, the authors said that glycogen was found in the decidua basalis in the early stages, and in the *Träger* in the later stages of development, but that there was no difference in the amount after crushing of the foetus or the injection of the hormone. Several methods had been used for the destruction of the foetus, but a simple puncture that released the amniotic fluid was usually sufficient to produce foetal death and resorption, though occasionally the puncture was repaired, the fluid reformed, and the foetus delivered at term. No evidence of malignancy had been seen. Young embryos were rapidly autolized *in situ*, older embryos persisted as debris in the uterus for a longer period, but could not be extruded if normal embryos blocked the way.

Dr R. Wheeler Haines read a paper on the 'Morphology of joints'.

It is usually stated that the interzone, the conspicuous layer of dense mesenchymal tissue separating embryonic cartilages, gives rise to all the tissues of the joints, including the capsule, ligaments and synovial tissues, as well as the articular surfaces. Examination of embryos of c.r. length 14–70 mm. shows, however, that the interzone forms the more central parts only of the articular surfaces, where the cartilages are in close contact. The capsule is a relatively late formation derived from the mesenchyme outside the inter-zone, and usually separated therefrom by a region of uncondensed 'synovial' mesenchyme, which later gives rise to the more peripheral parts of the joint cavity and to the synovial tissues.

This conception of the synovial joint as being composed of two distinct morphological entities, the interzone (originally a part of the blastemal skeleton) and the synovial tissues with the capsule which supports them (originally part of the surrounding mesenchyme) agrees well with the known evolution of joints. In fishes most of the joints are purely interzonal in type, and if a cavity develops it is in the interior of the interzonal tissue, as in the interpubic joint of man. In synovial joints the surrounding tissues take part in the development; the cavity appears first at the periphery and only later spreads into the interzone.

Professor Harris questioned the possibility of distinguishing cartilage and mesenchyme at such early stages, as the cartilage might still be growing at the expense of the mesenchyme.

Dr Whillis said that in developing newts there was movement between the cartilages in spite of anatomical continuity, and that the intervening matrix was of a special chemical composition and allowed this movement. He did not think the skeletal blastema could be identified in the early stages of joint formation.

The President considered that all joint tissues were derived from the blastema.

In reply, Dr Haines said that at the time in question the cartilages had already achieved their characteristic forms, and there was no evidence of the further participation of the general mesenchyme in their development in the human embryo. The cartilages were never united as had been suggested by earlier workers. The blastema seemed quite distinct and played no part in the formation of capsular or synovial tissues.

Dr A. Peacock reported two cases of 'Abnormal cervical vertebrae'.

(1) The fused atlas, axis and third cervical vertebrae from a case of generalized neurofibromatosis were described: the atlantal neural arch was defective and the occipital region presented unusual features.

(2) An anomalous atlas vertebra was described, wherein the vertebrarterial foramen divided the superior articular process into discrete moieties.

Professor Boyd commented on the congenital background of v. Recklinghausen's disease, and said that it was often associated with osseous variations, so that he had no doubt that the first specimen represented a congenital lesion.

Professor Cave said that the extra articulation in the second specimen might be due to a paramastoid process, and suggested that the specimens would be welcome in the Hunterian Museum.

Mr S. Wilson Charles read a paper on 'The control of accretion and absorption in membrane bones'.

It was shown previously that membrane bones are preformed in membrane and that ossification is a secondary process invariably preceded by interstitial growth of the membranous model until the definitive size and shape of the model is attained.

Examination of a series of heads from early embryonic life to adolescence shows (1) that accretion occurs on all surfaces and borders of the ossifying area of membranous models which are enlarging without alteration of curvature or form; (2) that areas of non-accretion and absorption occur in proximity to, or in association with, areas of accretion in models which are enlarging with alteration of curvature or form, and that some measure of accretion occurs on all ossifying surfaces even when alteration of curvature or form necessitates some associated absorption or non-accretion; (3) that very large areas of non-accretion, interspersed with small areas of accretion and absorption, occur over the ossifying areas of models which have attained (or almost attained) their definitive size and shape and are not altering in curvature or form to any appreciable extent. In certain instances (e.g., the parietal bone of the 24-week pig) one surface may have almost attained its full size, and show signs of non-accretion only, whilst the opposed surface may be in a state of active accretion.

Accretion is a characteristic feature of all surfaces of growing ossifying areas, and modelling absorption occurs only when there is alteration of curvature or form on the model. This suggests that accretion and absorption are controlled by the growth of the model, and that modelling absorption is in reality a process of de-differentiation due to a disturbance of equilibrium.

Dr Pritchard agreed with the statement that the growth of each membrane bone is preceded and determined by a membranous model, and said that adjacent models may meet end on, in which case each model grows by accretion, or obliquely, in

which case one model shows accretion and the other absorption of bone.

Professor BOYD also agreed with the conception of the model, which was shown clearly by Masson's stain, and said that mesenchyme cells, when cultured in the plasma of hens treated with large doses of parathyroid hormone, could give rise to osteoclasts.

Professor HARRIS commented on the large numbers of osteoclasts seen in tangential sections of the periosteal tissues where remodelling was in process, and in Paget's disease, but he did not think osteoclasts were essential for the removal of bone, since osteoblasts could either deposit or absorb bone.

Dr HAINES asked for the evidence that bone could be absorbed without osteoclasts, and commented on the lack of human material of the right age for the study of growth.

In reply Mr WILSON CHARLES said that where absorption could be demonstrated by measurement osteoclasts were not always present, and that when models overlapped accretion might occur equally on both the overlapping surfaces.

Dr E. W. WALLS gave an account of the 'Dissection of the human atrio-ventricular bundle'.

Considerable doubt has been cast recently upon many of the hitherto accepted facts regarding the specialized conducting tissue of the human heart.

To check certain statements regarding the impossibility of demonstrating clearly the human atrio-ventricular bundle by ordinary dissection, twenty human hearts were carefully dissected, with the following results. In all but two, some part of the bundle or its limbs was either demonstrated by dissection or else presented clearly beneath the endocardium. In three specimens the bundle and right limb formed so well marked a muscular band that photographs taken show the structures distinctly: photographs of this system in man are exceedingly rare, practically all the illustrations in the standard text-books being diagrammatic. The left limb of the bundle was not easy to dissect. Occasionally it showed through the left ventricular endocardium, but was so thin as to inhibit reflexions of the endocardium without damage to it. It may arise from the bundle as a number of branches coming off between the site of emergence of the bundle from the fibrous body and the site of its final division over the fleshy septum. Blocks were made from certain of the hearts and histological corroboration of some of the findings was obtained. The great difficulty experienced in tracing the bundle back to the node in some hearts was clearly due to the extremely dense character of the surrounding fibrous tissue of the heart skeleton.

Dissection of the atrio-ventricular node was not very successful. The artery to the node could frequently be traced into the mass of rather yellowish muscle but definition remained difficult, as might be expected from the histological structure of the nodal region as described by Blair & Davies (*J. Anat., Lond.*, 1935).

Professor KIRK, commenting on the difficulties sometimes experienced in exposing the bundle, said that he had found hyaline cartilage, whose nature he had confirmed by histologic examination, in the interatrial septum of the human heart.

Professor HARRIS inquired about the existence of a lymphatic or pseudo-lymphatic sheath around the bundle.

Dr WALLS said that there was no proper sheath in the human heart, and that was why the dye-injection technique failed to demonstrate the bundle.

The PRESIDENT spoke of the absence of the os cordis and of a true bacculum, preformed in cartilage, as human peculiarities.

Professor HARRIS read a paper by Dr D. V. DAVIES on 'The cell content of synovial fluid'.

In the past the content of nucleated cells in synovial fluid has been studied in a limited number of limb joints only, and consequently the erroneous idea has arisen that it is uniformly low. It is shown that the average normal count of nucleated cells in the synovial fluid of sheep and cattle varies greatly from joint to joint (from about 200 per cu.mm. in the limb joints to over 1200 per cu.mm. in joints such as the temporo-mandibular). The variation from joint to joint is probably related to the amount and character of the movement within the joint. Cell counts from costo-vertebral joints in cattle show low values in the joints associated with the relatively immobile first and second ribs. It is suggested that the cell count in any particular joint may vary with age in the absence of any degenerative changes. The cell count is highest in those joints with a highly viscous synovial fluid which are particularly free from degenerative changes. The presence of red cells in synovial fluid from a normal joint may be regarded as accidental. This explanation is suggested by the number, distribution and occasional complete absence of red cells.

In reply to Dr O'CONNOR, Professor HARRIS said that differential cell counts had been made, but there were large numbers of very small cells, so that identification was difficult.

Dr WHILLIS suggested that many of the cells were cartilage cells, and that the high viscosity of the fluid might be due to these cells.

Professor HARRIS replied that no cartilage cells had been seen, and that the viscosity of the fluid did not depend on its cellular content.

Dr W. F. Harper read a paper on 'The storage of vital stains (trypan blue, vital new red) in the rabbit endocardium'.

Vital staining of the rabbit mural endocardium, at non-toxic levels, proceeds similarly to that of the valvular subendothelium in this animal (Harper, 1943, *J. Anat., Lond.*, 77, 140). Macrophages are abundant and exhibit the property of segregating dye particles rapidly and in marked degree: their disposition as revealed by vital staining differs strikingly in atria and ventricles. In the former they occur throughout the entire depth of the endocardium, but in the latter are confined mainly to the endocardium proper, only a few vitally stained cells occurring in the subendothelium. Macrophages exhibit inequality in their ability to segregate dye particles and, irrespective of dosage intensity or period of administration, are always to be found alongside dye-laden cells and others morphologically similar but unaffected by dye. The significance of these findings is discussed. Undifferentiated connective tissue cells also acquire dye particles: the disposition of these is described and discussed. Odd vitally stained cells in the subendocardium are without significant relationship to the local blood vascular pattern.

Intracellular dye was observed both as a diffuse staining and as segregated cytoplasmic granular inclusions: their states are described and discussed. Endocardial cell reactions to certain experimental conditions are also described in the light of vital staining.

Dr Baxter asked whether diffusely staining cells were found, as he had noticed them in the suprarenal cortex and had interpreted them as dead or degenerate cells.

Professor Hamilton asked whether there was any correlation between the distribution of the vitally stained cells and the blood vessels, for he had noticed in the genital tract of the ferret that a large quantity of trypan blue was taken up when the uterus was in a highly vascularized state, but relatively little when in the atrophic state.

Professor Nicol asked whether the differences noticed between the distribution of the stained cells and the walls of the atrium and ventricle could be correlated with differences in the arrangements of the blood vessels.

In reply, Dr Harper said that there was no diffuse staining or other evidence of cellular death of cells in the endocardium within 2 hr. of the injection of the dye. The endocardium was, for the greater part, non-vascular, and the distribution of the dye, which probably permeated the wall directly from the heart cavity, did not depend on the vascular arrangements.

Dr J. J. Pritchard presented an account of his experiments on 'The repair of parietal bone fracture'.

The sequence of events during the repair of parietal bone fractures was followed in fifteen 1-year-old rats. Animals were killed at gradually increasing intervals up to 6 weeks, and their skulls examined histologically. Colchicine was injected as a routine 9 hr. before death, in order to increase mitoses.

New bone first appeared after 4 days within a layer of proliferating subperiosteal cells and blood vessels some distance from the fracture line. This reaction became very widespread until it covered the greater part of the skull, especially pericranially. Endosteal new bone was formed in slight degree. The region of dead bone and stripped periosteum in the immediate neighbourhood of the fracture showed no independent osteogenetic activity at any time. The events here were simply those usual in haematoma organization, namely, polymorph invasion, red cell phagocytosis, proliferation of fibroblasts and blood vessels, and foreign-body-giant-cell reactions about bony and other debris. No production of fibro-cartilaginous callus such as is described for other fractures was seen at any stage.

The debridement of the fracture line lasted a fortnight: then, while consolidation of the earlier formed distal bone occurred, the still proliferating osteogenetic cells invaded this region from the sides. The invasion was guided by the configuration of the repaired periosteum and the frayed edges of the dead bone. Bony union did not occur as no bony bridges were left to conduct the osteoblasts across the gap.

Finally, new bone formation ceased with lamellar consolidation of all the earlier formed cancellous bone. A thin layer of fibrous tissue united the edges of the original fracture.

Two conclusions from this study were: (1) the osteogenetic reaction was confined to the single layer of inconspicuous flattened cells lining the subperiosteal and endosteal surfaces of the bone; (2) in the absence of fibro-cartilaginous callus, the spread of osteogenetic tissue into the fracture gap was governed by the presence or absence of osteoconductive fragments of dead bone.

The President asked whether there was never complete union in the rat.

Dr Haines, commenting on the widespread subperiosteal reaction, extending over the opposite parietal bone, suggested that when one forearm bone became enlarged after the removal of the other, or one rib after the removal of its neighbours, this was due, not, as was usually maintained, to a compensatory hypertrophy in response to increased mechanical demands, but to a subperiosteal response to a chemical stimulus from broken bone or blood clot.

Dr WALLS asked how, in Macewen's classical case, fragments of broken-up human parietal bone placed on the dura could survive.

Professor HARRIS asked about the comparative degree of callus formation under the periosteum and under the dura.

Professor CAVE commented on the naturally occurring fractures found in animals, and asked if a saw cut remained straight or became eventually wavy and suture-like.

Dr WHILLIS said that fibrous union occurred in membrane bone fractures unless bone grafts crossed the gap, and this was the reason for the use of a sliding graft made of a single table of the skull. He inquired as to the source of the new bone where the periosteum had been stripped.

Professor KIRK asked whether the bony thickening persisted as an end result or whether the callus was absorbed with a lapse of time.

In reply Dr PRITCHARD said that after removal of a piece of parietal in the rat there was no bony union after 77 days, and this was a long time in the life of a rat, but that single saw cuts could heal over, and while doing so remained straight. The old callus was not absorbed and osteoclasts had not been seen in association with it. The widespread subperiosteal reaction depended on the presence of blood clot, for there was little reaction when clot was absent. Human parietal bones are richly vascularized, while rat parietals had but few vessels, so the behaviour of isolated fragments might be quite different in the two species. Subperiosteal callus formation was more intense than subdural, which was absent except where the dura had been stripped. New bone did not arise from stripped periosteum, but from intact periosteum beyond the region of stripping.

Professor A. B. APPLETON read a paper on 'The interlobar approach to the roots of the lungs'.

In both lungs the lower lobe pulmonary artery separates the main bronchi from the pleura of the oblique fissure.

In the right lung the vascular pattern displayed on approach through the oblique fissure varies greatly according to differences in the size and arrangement of the arteries and veins of the posterior segment of the upper lobe. In the arterial type of arrangement numerous arteries are seen to take divergent courses from their origins on the main artery to supply the posterior upper lobe segment, the middle lobe and the dorsal segment of the lower lobe. In the venous type of arrangement the arteries to the posterior segment are inconspicuous and are concealed from view by large veins that drain the posterior upper lobe segment or the lower lobe dorsal segment, or both: on opening the horizontal fissure the most conspicuous structure is the great upper lobe vein.

In the left lung variations in appearance depend on differences in the site of origin of the artery to the pectoral segment of the upper lobe, which may arise anteriorly to the root or low behind it. In the former arrangement the upper lobe is readily separated from the lower lobe artery on interlobar approach. The latter, and commoner, arrangement, complicates a left upper lobectomy, since thereby the arterial supply of the lower lobe is endangered.

In reply to Dr WALLS, Professor APPLETON said that his terminology was based on convenience, and that all the terms suggested, such as pectoral, apical and posterior, had been used by surgical workers.

Professor WEST and Professor CAVE commented on the variation seen in the blood vessels of the bronchopulmonary segments and the surgical difficulties involved in their removal.

In reply to Professor KIRK, who asked whether arteries and veins could be distinguished in the living lung, Professor APPLETON said that this was difficult, and depended on recognizing the anatomical patterns, and so on knowing what patterns to expect.

Professor HARRIS said that veins usually lay in positions where there was a maximum of loose areolar tissue, and that this principle explained the presence of the superior vena cava on the right side, and the supersedure of the cephalo-jugular trunk by the axillary vein. In the lung the veins lay as near the periphery of the lobules as possible or in the loose interlobular connective tissue.

The PRESIDENT suggested that some of the variations might be apparent rather than real, being due to local venous engorgement, but Professor APPLETON replied that the variations were real, and that there was a reciprocal development observable between the various venous pathways.

Dr Z. MENSCHIK-MYSZYNSKI, introduced by T. ROGALSKI, read a paper on 'The influence of vitamin E on ovarian structure in mice'.

A serial histological study has been made of the ovaries of mice on several different diets from day of weaning, the principal being a vitamin E-deficient diet and one containing α-tocopherol ('Ephynal' Roche) as the only vitamin E source. Control animals were kept on stock diet, lettuce being the main source of vitamin E. Animals were killed after periods ranging from 30 to 656 days of particular diet.

Animals on vitamin E-rich diet, compared with controls, show ovaries with more primordial ova and primary follicles: follicular cells and interfollicular tissue of epithelial origin are more abundant, but degenerating follicles are numerous:

there are fewer mature follicles and corpora lutea, both of which are enlarged.

After prolonged vitamin E-rich diet the ovary is small with degenerating follicles containing blood: neutral fat is present in the ovarian medulla and adipose tissue in the mesovarium is extremely abundant.

Animals on vitamin E-deficient diet, compared with controls, show ovaries with fewer primordial ova and less interfollicular tissue: follicles contain fewer follicular cells; corpora lutea are increased, but are smaller and of various ages; medullary lipids, not readily soluble in fat solvents, are prominent, yet there is complete absence of neutral fat in both ovary and mesovarium. As the vitamin E deficiency continues, the lipids invade the ovarian cortex.

After prolonged vitamin E-deficient diet the ovary, comparatively large, is hard and yellowish and contains only cells filled with lipids other than neutral fats.

The ovarian changes in this study appear to be quantitative rather than qualitative; but it seems certain that vitamin E stimulates the development of all elements of germinal epithelium origin, and influences fat metabolism in the ovary and its neighbourhood so that neutral fats increase, while the formation of other lipids is inhibited.

In reply to Professor NICOL, who asked if any special thickening of the germinal epithelium in mice on a vitamin E-rich diet had been noticed, Dr MENSCHIK said that there were more mitoses than usual, but no change in thickness.

Dr PRITCHARD said that the work showed that in the mouse the effects of vitamin E were equivalent in the ovary and testis, whereas in the rat there was no such correspondence.

Professor W. J. HAMILTON read a paper by the late Dr D. M. SAMUEL on 'The use of an agar gel in the sectioning of mammalian ova'.

Small objects such as mammalian ova are embedded in an agar gel. This can then be carried through all the stages of dehydration and clearing and, while in methyl benzoate, the ova may be examined through the agar under an oil-immersion lens. Nuclear detail may be determined and orientation for sectioning decided.

Professor W. J. HAMILTON read a paper on 'The first findings of a pronuclear stage in man'.

An unsegmented ovum was recovered from the uterine tube and photographed in the living state. The measurements were: outer diameter of zona pellucida 173 μ; inner diameter of zona pellucida 135 μ; diameter of vitellus 100 μ. After fixation, etc., the ovum was cut serially at 7 μ. The cytoplasm was uniformly granular and contained two pronuclei, one of which was larger than the other. Many sperms were found in the zona pellucida.

Professor W. J. HAMILTON, with Dr F. T. DAY, read a paper on 'The segmentation stages of the horse ovum'.

Some stages of the segmentation of the ovum of the horse are described. The time of ovulation and its bearing on artificial insemination is discussed.

Measurements are given of the different stages.

Dr A. D'A. BELLAIRS and Professor J. D. BOYD gave their 'Observations on the lachrymal apparatus in squamata'.

The relations of the orbital (Harderian) gland, the sub-conjunctival space and the lachrymal duct system were investigated in seven lacertilian and in eleven ophidian species. In all of the lizards investigated the orbital gland discharges by separate ductules into the conjunctival (or, when present, the sub-brillar) space. This space is drained by two puncta situated on the deep aspect of the lower lid and the resulting canaliculi unite to form a lachrymal duct. This relationship is not influenced by the replacement of the lids by a brille. In most snakes the orbital gland discharges by a single duct into the upper end of the lachrymal duct and not into the sub-brillar space directly. The lachrymal duct itself arises from the sub-brillar space by a narrow canaliculus. Certain primitive snakes (*Typhlops*, *Boa* and *Aenopeltis*), however, depart from this usual ophidian arrangement and show transitional stages between it and the usual lacertilian condition.

Professors W. J. HAMILTON and J. D. BOYD made a contribution on 'The early development of the mesoderm in the sheep'.

The method of origin and spread of the mesoderm and its early differentiations are described. Special attention is directed to the primitive streak and to the early differentiation of the intra-embryonic coelomic epithelium. The results are based on the study of an extensive series of sheep embryos of the age period from the 6th to the 35th day of gestation.

Lack of time prevented any discussion on the last five papers.

JUNE 1945

The Summer Meeting of the Society was held in the Anatomy Department of the Victoria University of Manchester on Friday and Saturday, 29 and 30 June, beginning at 10 a.m. each day, with the President (Professor WOOD JONES) in the Chair.

Dr E. L. PATTERSON read a paper on 'The morphology and function of mammalian digital sesamoid bones'.

The form and distribution of digital sesamoid bones have been examined in representatives of a number of mammalian orders. The view is expressed that the digital sesamoid is primarily a capsular strengthening to protect a joint from trauma without limiting its movement.

In general, mammals retain sesamoids at those digital joints which are subjected to pressure. In man, the basal digital sesamoids are most frequent at the pressure-bearing extremities of the transverse metatarsal and metacarpal arches.

The attachment of a tendon to a digital sesamoid is a secondary affair, but assists the function of the joint by facilitating proximal excursion of the sesamoid during flexion.

In its primitive form, a digital sesamoid is a single plate or bar in the joint capsule. At the basal digital joints it becomes enlarged on each side of the flexor tendons, assuming a dumb-bell shape. This arrangement relieves from pressure the tendons which flex the interphalangeal joints, so that the digits can maintain grip while the manus and pes are taking weight. The waist of the dumb-bell reverts to cartilage since twin sesamoids can move independently and are, therefore, more satisfactory at joints permitting lateral movements.

In reply to Professor DAVIES, who asked if the sesamoids were associated with the tendons in the embryo, Dr PATTERSON said that bursae are sometimes found between the tendons and the sesamoids, so that they must be independent structures.

Dr HAINES questioned the advisability of using the monotreme *Echidna* as the starting-point of a morphological series, and suggested that dumb-bell-shaped or twin sesamoids might be more primitive than bars, since special arrangements were often developed to protect tendons from pressure which would occlude their blood vessels.

The PRESIDENT pointed out that the sesamoids described by Dr PATTERSON on the dorsal aspect of the digits of some burrowing animals supported his contention that the sesamoids protected the joints.

Dr J. H. SCOTT read a paper on 'The early development of the enamel organ'.

An examination has been made of the developing enamel organ in human embryos of 30 mm. or more in length. This examination confirms the presence of the enamel niches of Bolk (*J. Anat., Lond.*, 1920–4), but it is suggested that these have not the significance attributed to them by that author. Rather, it is held that they are but part of a general process whereby that portion of the dental lamina giving origin to the enamel organs of the permanent teeth is separated from the lingual side of the enamel organs of the deciduous teeth.

In reply to Professor DAVIES, who asked if the processes described applied to all the teeth, as Bolk's theory applied to the multicuspid teeth only, Dr SCOTT said that they applied to all the deciduous teeth, including both incisors and molars.

Dr J. J. PRITCHARD read a paper on 'Osteogenetic stimuli'.

Some of the stimuli capable of initiating and inhibiting the production of new bone have been investigated in adult rats. Fresh heterogeneous tissues were implanted under the scalp, without damaging the subjacent cranium or pericranium. At intervals afterwards the heads were sectioned and the responses of the skull and scalp noted. Adult spleen and testis when so implanted stimulated rapid proliferation of subpericranial osteoblasts after a latent period of 2 days. After 4 days reticular new bone appeared, and increased to a maximum after 7 days: it later became consolidated by the addition of perivascular and surface lamellae. Simultaneously the vascular canals of the original bone were enlarged by osteoclastic resorption. No new bone appeared within the diplöe or endocranially. Boiling the splenic implant destroyed its ability to stimulate new bone production.

With other tissues employed the response was slight or absent unless infection occurred, when bone resorption was marked in the immediate vicinity of the implant, but distant bone production was stimulated. The new bone was somewhat less thick where the implant exerted direct pressure on the skull. It is concluded that diffusible stimulants to osteoblastic proliferation are produced during the autolysis of splenic and testicular implants, that infected tissues liberate substances which stimulate osteoclasts locally and osteoblasts more remotely, and that direct pressure inhibits new bone production.

The sequence of events after splenic implantation is almost identical with that following fracture of the skull, and is suggestive of the operation of similar humoral factors.

Dr P. BACSICH suggested that the activity of spleen and testis implants was due to their high hyaluronadase content.

In reply to Dr GLEES, who asked whether a combined implant of spleen and cartilage would lead to a premature ossification of the cartilage, Dr PRITCHARD said that his experiments were made on the intact skull so as to avoid any complication by mechanical factors.

In reply to Professor YOFFEY, who asked whether there was any migration of splenic pulp cells from the implant, Dr PRITCHARD said that the capsule of the implant remained intact, and no cells got out from the implant.

Dr SCOTT commented on the increase of bone in bird skulls in chronic tuberculous disease.

Dr R. W. HAINES read a paper on 'Pronation and supination in tetrapods'.

On palaeontological evidence the early tetrapods appear to have developed a mechanism of pronation and supination similar to that in the more primitive extant reptiles (*Sphenodon*, some lizards and turtles). The radius and ulna articulated with the humerus and with each other at the elbow, but diverged distally to articulate separately at the wrist, being unconnected by any interosseous ligament. In pronation and supination (totalling about 45°) the manus rotated on both radius and ulna, which both rotated on the humerus, so that rotation occurred partly at the wrist, partly at the elbow.

In typical mammals (e.g. *Dasyurus*, *Didelphys*, *Felis*, *Sciurus*) the radius and ulna are closely disposed and are united by a strong interosseous ligament. Movement between the bones now takes place along an axis passing through the radius, and when it amounts to about 90° an annular ligament is developed. Both radius and ulna still rotate on the humerus, a complex series of ligaments being consequentially developed. (Urodeles, crocodiles, birds and monotremes possess specialized mechanisms, these groups not illustrating primitive conditions.)

In Primates the radius and ulna have become bowed away from each other, the interosseous ligament has widened, and the range of movement has increased to nearly 180°. The more primitive forms (e.g. *Cebus*) preserve the typical mammalian ligamentous arrangement and the ulno-humeral rotation, but in more specialized forms such as man these features have been lost. Whilst man has inherited the general character of the movements of pronation and supination from remote tetrapodal ancestors, the range and the mechanism of these movements are of relatively recent acquisition.

In reply to Professor DAVIES who asked whether the complex system of ligaments found in typical mammals could be concerned with the establishment of a synarthrodial position, Dr HAINES said that he had found no evidence of such a position, for as one ligament was wound in by rotation of the radius the other was at the same time played out.

In reply to Dr FYFFE who asked whether rotation of the ulna occurred in man, and how recently it had been lost, Dr HAINES said that in man rotation was detectable but negligible in amount (about 2°) and that, as far as could be judged from living animals, this loss was relatively recent, as the movement is still found in old-world monkeys.

A short general discussion on pronation and supination followed.

Dr W. C. OSMAN HILL read a paper on 'Nasopalatine canals in the living adult'.

No definite record exists of complete bilateral persistence of the nasopalatine canals in the human adult. Partial patency of one canal or of both canals, most commonly at the nasal end, has been recorded from cadavera. Complete bilateral patency of the canals is here reported in a young adult Englishman, who noticed that he could elicit palatal noises by sucking hard with closed mouth, accompanied by injection of cold air through the region posterior to the incisor teeth.

The introduction of coloured dye into the nasal fossae, followed by sucking with the mouth closed, brought the dye into the mouth. Investigation revealed minute slit-like orifices, partly concealed by the incisive papilla, $\frac{1}{2}$ in. behind the maxillary incisors. The papilla had a valvular action preventing oro-nasal passage of fluid. Wires were passed through the canals for purposes of radiography, the right canal proving the wider. Radiographs show the anatomical relations of the canals. Rhinoscopy revealed no evidence of Jacobson's organ, possibly because of an earlier submucous septal resection. The importance of the canals, or their vestiges, in the aetiology of cysts of the premaxilla is stressed.

Dr W. BRANDT commented on the practical importance of epithelial cysts in the region of the incisive fissure.

In reply to Professor HARRIS, who asked if there was a family history of hare lip or cleft palate, Dr OSMAN HILL replied that there was no such history.

The PRESIDENT commented on the difference in the quality of the sensation elicited by rubbing the tip of the tongue over the incisive papilla as compared with the rest of the palate.

Dr W. BRANDT read a paper on 'Experimental production of supernumary humerus in amphibian embryo (*Amblystoma mexicanum*)'.

In stage 35 (Harrison) of the embryo, a piece of ectoderm cut from the tail of another embryo was implanted deep into the tissues of the limb bud. Three weeks after operation serial sections were made from the larvae and a reduplicated humerus was found in two out of 63 cases. In each instance

the implanted ectoderm extended throughout the larval forearm, but its proximal extremity terminated in the distal end of the humerus.

The proximal end of the head of the humerus showed a thickening, the distal end was oval, and in the area of the neck an hour-glass contraction was visible. Distal to the neck, the shaft of the bone was duplicated and the upper ends of the shafts were united by a small bridge of cartilage, but more distally they were entirely separate. The shafts had a rounded outline. One duplicated humerus was attached to the forearm, the other ended by a pointed distal extremity which lay free in the tissues of the body wall.

This is the first experimentally produced reduplication of a limb-unit (the humerus).

Presumably the primordium of the humerus was split by the implanted ectoderm, and this splitting resulted in a 'twinning' of the primordium which, in stage 35 of the embryo, is in the 'reversible phase of determination' (Brandt, 1923). Each half of the split primordium developed an entity in accordance with the potencies of that phase of determination.

In reply to Dr O'Connor, who suggested that the conception of determination had been strained and that the phenomena described might be due to regeneration, Dr Brandt said that determination is a physiological term indicating a process whose mechanism we do not know, but of which we see the effect, while differentiation is an anatomical term, and that since in his experiments there had been no destruction of tissue there could be no question of regeneration.

In reply to Dr Wyburn, who commented on the similarity between the results of experimental interference and the action of pathological genes, Dr Brandt said that identical types of deformity can be produced by these factors.

Drs P. Bacsich, A. Sharman and G. M. Wyburn presented a paper on 'The reputed hormone of semen'.

Recently Green-Armytage (1943) published the results of experiments from which he concluded that semen possesses a hormone allied to testosterone, which he states to be of 'tremendous importance' to the female genital tract, particularly in the early months or even years of matrimony. His experiments—the injection of semen into immature rats and rabbits—have been repeated and further extended by similar treatment of immature and spayed adult guinea-pigs, together with a control series treated with testosterone propionate. The results, which on the whole do not confirm the findings of Green-Armytage, are discussed.

In reply to the President and Professor Hamilton, who questioned the validity of experiments involving the injection of the products of one species into the other, in related species, it was stated that the object of the experiments was to repeat the work of Green-Armytage as it had originally been carried out.

In reply to Drs Pritchard, Glees and Bacsich, who commented on hormone actions, it was stated that, so far as could be judged from the experiments described, the semen contained no hormone at all.

Dr J. S. Baxter read a paper on 'Cellular growth and degeneration in the rat adrenal cortex'.

The processes of cell formation and degeneration have been studied in the adrenal cortex of the adult rat, stained with intravitam trypan blue.

The majority of the cells of the capsule and of the subcapsular (glomerular) regions react to the dye in a uniform manner, segregating it in the form of very fine cytoplasmic particles. This process commences during the first 24 hr. after injection of the dye and is marked at 48 hr. Cells in the outer part of the zona fasciculata also segregate dye particles: these are not so evident, and are mainly located towards one pole of the nucleus.

Three types of cell in the zona reticularis take up trypan blue: (1) the 'dark' cells, which usually also contain pigment granules; (2) macrophages; (3) dead or dying cells. The significance of these cells is discussed.

Mitotic counts for each zone of the cortex have been made for a series of normal animals, and also in a second group to which colchicine had been administered. The results obtained are discussed with reference to the theories of adrenal cortex growth.

In reply to Dr Harper, who asked whether the cortex had been examined at different periods and whether there was any change in the dead cells, Dr Baxter said that his preparations were made from rats taken from 22 hr. to 21 days after the last injection, and that there was little change in this period, but that the dead cells disappeared.

Dr Bacsich commented on the x and y zones of the cortex.

Professor J. M. Yoffey and Dr J. S. Baxter presented a paper on the 'Pituitary, adrenal cortex, and lymphoid tissue in the rat'.

The mechanism for the control of the number of cells in the blood stream constitutes a fundamental problem in haematology, concerning which but scanty information exists.

Recently, experiments have been reported indicating that the lymphoid tissues are controlled by the adrenal cortex, which, in turn, is under the influence of the adrenotropic hormone of the anterior lobe of the pituitary.

In the present communication experiments on rats are reported to show the effect upon the thymus

and lymphoid tissues, the blood lymphocytes, and the adrenal cortex of (*a*) pituitary adrenotropic hormone (cortrophin, organon), (*b*) extract of adrenal cortex (eschatin, Parke, Davis & Co.), (*c*) both cortrophin and eschatin in conjunction.

In answer to Dr O'Connor, who asked if the effects observed could be due to changes in blood volume, it was stated that there were probably no significant changes, and in answer to Dr Bacsich, who asked if the work had any application to the treatment of lymphatic leucaemia, that in human cases softening of the lymph nodes had occurred after endocrine therapy, but that the effect was not lasting. In animals there was tumour destruction and the animals lived longer, but clumps of resistant lymphocytes survived, and the reticular cells regenerated malignant lymphocytes.

Professor T. Rogalski and Dr O. Rymaszewski presented a paper on 'Crossed and uncrossed optic fibres of the dog'.

Investigation was made of a case of left-sided ocular cystic degeneration in a dog, wherein all optic nerve fibres on the diseased side had undergone complete degeneration. The right optic nerve, and both optic tracts, were stained by the silver technique, and the numbers of axons were estimated in strictly transverse sections thereof. Axon counts were: right optic nerve, 50,099; left optic tract (crossed fibres), 39,405; right optic tract (uncrossed fibres), 8,780. Thus 82 % of optic nerve fibres cross in the chiasma, and 18 % remain uncrossed, the average rates of crossed to uncrossed fibres being 4·5 : 1.

The surface areas of the sectioned nerve and tracts were also measured and compared. The left optic tract measured 2·8 sq.mm. and the right 0·6 sq.mm., the ratio being much the same. Hence area measurements of optic tracts are of value in estimating the proportions of crossed and uncrossed optic fibres.

In answer to Dr Glees, who asked about the relation of myelinated and non-myelinated fibres, it was stated that the sections were stained with silver, so that only the axons were seen.

Professor A. Durward read a paper on the 'Experimental investigation of the intraneural vascular bed of peripheral nerves'.

A study has been made of the relative importance of the intraneural longitudinal vascular channels and the regional sources of blood supply to a nerve. It has been suggested that the regional sources dominate their own fields of supply, but experimental evidence (Adams, 1943, *J. Anat., Lond.*, 77) has not given entirely unequivocal results. The present investigation was planned to elucidate the part played by the intraneural vascular bed disposed along the length of the nerve. The results of experiments on the rabbit's sciatic nerve suggest that the most important factor in maintaining an adequate circulation within the nerve is the integrity of the longitudinal channels. The discrepant results in previous experimental work are discussed.

Drs R. Barnes, P. Bacsich and G. M. Wyburn presented a paper on 'A histological study of a predegenerated human nerve autograft'.

An autograft taken from the distal end of an ulnar nerve which had suffered an irreparable injury was sutured to the central stump and removed 5½ months later for histological examination. The central stump retained its regenerative capacity 613 days after injury. The autograft survived and provided a pathway for new nerve fibres, although none of these fibres was medullated. The absence of medullation may have been due to extensive intrafascicular fibrosis, which prevented normal maturation of the nerve fibres. There was no central necrosis of the autograft.

In answer to Dr Glees it was stated that the axons in the graft were thinner than in the normal nerve, that changes in the endoneural sheaths could not be made out as the fixation was poor, that no motor end-plates were seen, all nerve endings being free, and that no action currents had been taken, and, in answer to the President, that a pathological report on a control piece of the autograft at the time of implantation showed the absence of nerve fibres at that time. Dr Pritchard suggested that as it had been shown in the cat that sympathetic nerve fibres might grow anywhere, the fibres seen in the graft might be sympathetic in nature.

Dr G. W. Harris communicated 'Some data concerning the innervation of the hypophysis'.

The remote-control method of stimulation has been used for studying the innervation of the neurohypophysis in the conscious rabbit. The stimulus used was well localized, the effective current spread being about ½ mm. Stimulation of the median eminence, infundibular stem and infundibular lobe produced an antidiuresis, chloruresis and uterine contraction. Stimulation of the caudal part of the tuber cinereum, the ventro-median hypothalamic nucleus and the region of the paraventricular nucleus failed to give these reactions. It seems unlikely that the fibres described as arising in these latter structures and entering the infundibular stem are concerned with the secreto-motor innervation of the neurohypophysis. The oxytocic activity appears to be under the control of the same tract as the antidiuretic, viz., the supra-optico-hypophysial tract.

In answer to Dr Glees, who suggested the removal of both eyes, Dr Harris said he preferred not to

interfere so seriously, and to Professor YOFFEY, who asked if his work confirmed the general conception of the results of hypothalamic stimulation, that he had insufficient data on blood pressure and intestinal movements to be able to draw a conclusion.

Drs P. GLEES, A. MEYER and M. MEYER read a paper on 'Terminal degeneration in the cerebral cortex following the interruption of afferent fibres'.

Following observations on bouton-like structures in the normal cerebral cortex, a study has been made of the changes which these undergo after experimental lesion in the rabbit brain. The significance of these changes is discussed, as also the application of this method to the study of the site of terminal distribution of various afferent fibres to the cortex.

Professor JEFFERSON said that in human frontal lobe operations it was difficult to know just what had been done, and that he would prefer to work with a bone flap instead of a trephine hole. He asked about the method used for orientation. In reply it was stated that a large exposure of the brain surface was made, and the motor area explored electrically, and used as a landmark, as otherwise orientation was impossible.

A general discussion on the effects of frontal lobe injuries followed.

Dr E. J. FIELD read a paper on the 'Early development of the heart in the sheep'.

The earliest development of the sheep heart was investigated in a series of embryos of known age. There was no evidence of a 'cardiogenic' plate in the floor of the earliest coelomic clefts at the cephalic end of the embryo. The earliest vessels appeared as vesicles close against the endoderm, and these were connected by strands of nucleated protoplasm constituting 'angioblastic strands'. There were no well-defined endothelial tubes prior to the formation of the head and lateral folds. The closest relationship existed between the earliest vessels and the endodermal floor of the foregut.

In answer to Professors WEST and HAMILTON, who commented on the difficulty of deciding the origin of the angioblasts, Dr FIELD said that the germ layer theory needed revision. In answer to Dr G. I. BOYD he said that a ventral mesocardium was formed by the fusion of coelomic folds.

Dr F. P. REAGAN read a paper on 'Human foetal cardio-vascular arrangements'.

The eighteenth-century anatomists established the distinction between right atrium and vena cava inferior and a definition of the cava still pertinent to any study of foetal circulation. Recent physiological findings regarding the foetal cardio-vascular system are capable of adequate description without recourse to a neo-Galenic terminology liable inadvertently to obscure or to discount essential differences between cardiac and vascular structures. The plan of the foetal circulation as outlined by Barclay, Franklin, Prichard *et al.* is discussed. Observations are made on persistent sinus venosus valves in a 133 mm. human embryo and on the so-called ligament of the left superior vena cava. A plea is made for the adoption of a terminology for the foetal vascular system which shall be uninfluenced by embryonic or post-natal arrangements.

The PRESIDENT and Professor CAVE urged the publication of Dr REAGAN's fine drawings.

Mr V. F. LAMBERT read a paper on 'Pterygoid extensions of the sphenoidal sinus'.

Among the many recognized variations of the sphenoidal sinus is that wherein it invades the pterygoid process. Such pterygoid extensions are of clinical importance. Their radiographic appearance and their anatomical relationships are described.

In answer to Professor DURWARD, who asked if the sinuses involved the lesser wing, as he had seen them do in the dry skull, and what was known of the development of the sinuses, Mr LAMBERT said all his material was based on living subjects, and he had not seen the extension mentioned, and that the sphenoidal air sinuses began their development in the 6th year and might continue to grow in adult life.

A general discussion on the extensions of air sinuses and their limitations followed, the PRESIDENT, Professor HAMILTON, Dr PRITCHARD and Dr HAINES taking part.

Dr J. WHILLIS showed a film illustrating the 'Movements of the tongue'.

A ciné-film record is presented of the movements of the human tongue, in the case of a patient in whom the left cheek had been removed at operation.

In answer to Professor DURWARD, who asked if the epiglottis was bent down and came up again slowly, Dr WHILLIS said that he had not seen it. The movement was probably passive, dependent on the upper end of the epiglottis being caught by the bolus, so that when there was no bolus it did not occur, but there might be individual variation. Professor HARRIS asked whether the subject's speech had been affected by the large defect in the cheek which allowed the film to be taken, and Dr WHILLIS answered that the mechanism for the formation of consonants seemed normal, and that speech was intelligible but altered.

Dr P. GLEES read a paper on his 'Observations on the injured pyramidal system of the cat'.

Animals operated on in collaboration with Professor LIDDELL during the last three years

permitted an anatomical study of degeneration in this descending fibre system. The following experiments were carried out: (*a*) section of the pyramid at pontine or medullary level; (*b*) removal of the motor cortex only; (*c*) removal of motor cortex followed by pyramidal section; (*d*) removal of motor cortex after pyramidal section. The composition of pyramidal fibres, the mode of degeneration of the myelin sheath and axon, and glial reactions, have all been studied at different time intervals.

Dr STEWART commented on the early changes in the axon which he had seen in the pulp of teeth affected by chronic irritation. Professor GOLDBY said that in the Australian opossum, after the extirpation of the motor cortex, many fibres of the pyramid survived, and these had their origin in the striatum. Dr GLEES said that in the rabbit it was necessary to remove the whole hemisphere before all the fibres were destroyed, for the cortico-spiral fibres formed only a component of the pyramid.

In reply to Dr WYBURN, who asked whether the animals learned to carry out complicated movements after extirpation of the cortex, as the sheep learned to walk over hillsides and rocks, Dr GLEES said that in the cat the mechanism for the finer skilled movements lay in the cortex, and the cat was unable to readapt itself when the cortex was lost.

Dr G. F. HAMILTON read a paper on 'The longitudinal muscle coat of the human colon'.

Contrary to accepted views, the colon possesses a complete outer longitudinal coat, probably as important as the undisputed outer coat of the small intestine. This longitudinal coat, in the intervals between the taeniae, is a little less than one-half the thickness of the circular coat.

The relative amount of the longitudinal muscle located in the taeniae seems fairly constant in the ascending and transverse colon, but in the descending and in the pelvic colon the taeniae begin to increase at the expense of the rest of the outer longitudinal muscle coat. In the pelvic colon the total bulk of the longitudinal muscle begins to increase.

Radially arranged smooth muscle fibres are described, passing from the longitudinal coat through the circular coat towards the submucosa.

It is suggested that the colonic sacculations may be due to the circular muscle coat.

Professor J. KIRK read a paper on 'Further observations on the histology of the terminal portion of the common bile and pancreatic ducts'.

The presence of a nerve supply to the sphincter muscle has been investigated, and fibres supplying the muscle surrounding the junctional zone of the ducts in the submucous coat of the duodenum are demonstrated. There is also anatomical evidence that secreto-motor fibres are distributed to the mucous glands which are so abundant in the walls of both ducts. The presence of pancreatic tissue embedded deeply in the wall of the duodenum is not so rare an occurrence as might be supposed.

In answer to the PRESIDENT and Dr GLEES, who asked about the distribution of islets and the possibility of distinguishing pancreatic tissue from Brunner's glands, Professor KIRK said that no islets had been noticed, but that the general appearance of the tissue differed from that of the glands. In answer to Dr FIELD and Professor CAVE, who commented on the action of drugs such as morphine given hypodermically or amyl nitrite nasally, and asked how much muscle there was in the common bile duct, Professor KIRK said that he had only examined one specimen. The longitudinal muscle of the duodenum turned back on the duct, ran along its wall for some distance, and then faded out, but the greater part of the duct had no muscle in its wall.

Professor A. DURWARD and Dr K. M. RUDALL presented a paper on 'Factors influencing hair-growth rhythm'.

In the rat, hair growth on the trunk occurs in waves proceeding from the mid-ventral to the mid-dorsal line. A more complicated pattern governs hair growth on limbs and head. In this zone of active growth, and keeping pace with it in its dorsal progression, there is a pronounced increase in vascularity as evidenced by staining and injections. Experimental investigation is being made into factors which may influence or control the behaviour of the growth wave: e.g. nervous, vascular and humoral. The histology of the vascular bed within the zone of growth and at its advancing and receding edges is being studied.

Dr OSMAN HILL commented on the inhibition of hair growth during mammary activity, particularly in *Loris* and *Nycticebus*, where it disappears over the mammae, and suggested that there might be two sets of follicles, one active and one passive in any particular cycle. In reply it was stated that the time cycle between successive waves of hair growth was about six weeks, but might be ten weeks in badly fed animals. All the follicles in the area of activity were active, and each hair persisted for three or four cycles before it fell out.

The PRESIDENT suggested a study of the shrew as a moulting animal, and Professor HARRIS pointed out that the waves of growth bore no relationship to the dermatomes.

A general discussion on the regulation of hair growth followed, Drs WHILLIS, HAINES and BACSICH and the PRESIDENT taking part.

Dr I. W. Monie read a paper on 'The intra-abdominal portions of the foetal umbilical arteries'.

The growth and form of the subendothelial cushions found in the intra-abdominal portion of the umbilical arteries have been examined in embryos ranging from 30 mm. to full term. These subendothelial cushions form ridge-like projections into the lumen which may run parallel or obliquely to the long axis of the vessel. They are composed mainly of longitudinal and oblique muscle fibres. No subendothelial cushions are present in the 30 mm. embryo, but at the 67 mm. stage a few small cushions are apparent. As the embryo grows, these subendothelial cushions project into the lumen and become more prominent, and in the older embryos they occur throughout the course of the intra-abdominal portion of the umbilical arteries. The subendothelial cushions are considered to play a part in controlling the vessel lumen and in closure of the umbilical arteries at birth.

Dr Wyburn said that the early appearance of the cushions suggested that they must perform some useful function during the course of embryonic life, and not only at its close.

In answer to Dr Glees, Dr Monie said that he had not investigated the nerve supply of the arteries, but that when perfused with oxygen-rich blood they contracted, and opened with blood rich in carbon dioxide, and suggested that the umbilical arteries cope with the large variations. Dr Bacsich commented on the cushions of the extra-embryonic portions of the arteries.

Dr P. Bacsich read a paper on 'Heparin in the placental circulation'.

Grosser viewed the slowing of circulation in the intervillous spaces of the human placenta as a phylogenetic advancement towards a greater efficiency of placental function. Others expressed the view that the blood in the intervillous spaces tends to clot even in the normal placenta.

By special staining methods the presence of heparin, or some other chemically related substance, was demonstrated both in the decidual part and in the chorionic plate of the human placenta. It is thought that this physiological anticoagulant may play a part in the maintenance of the circulation of the intervillous spaces, although it is felt that these results, obtained by histological methods, will require further chemical analysis. Human and various animal placentae were examined. Certain clinical aspects of the obtained data are discussed.

Professor J. D. Boyd and Dr W. F. Harper presented a paper on 'The development of the cavernous tissue of the human nasal mucosa'.

The venous spaces of the nasal mucosa have been studied in a large number of human embryos, foetuses and new-born infants, and of children in the first decade of life. Venous dilatation to form cavernous tissue occurs early (44 mm. stage, c.r. length), and is extremely well developed in 150 mm. foetuses. The structure and connexions of the vascular spaces in the nasal erectile tissue are discussed.

Dr D. V. Davies read a paper on 'The lymphatic vessels of synovial membrane'.

The lymphatic vessels of the synovial membrane have been studied in the metacarpophalangeal and metatarsophalangeal joints of recently slaughtered cattle by the stab technique with 50% India ink. Histological sections prove that the injected vessels are lymphatic channels. The arrangement and characters of the lymphatics in the synovial membrane and their connexions with extra-articular lymphatics were traced. Contrary to the views of some investigators, injections of India ink directly into the joint cavity followed by massage and movement failed to fill the synovial lymphatic plexus. In several cases the stab injections also entered the blood vessels as well as the lymphatic vessels.

In reply to Dr Walls, Dr Davies said there was no need to use putrified material as young fresh material gave 90% of successes.

The President commented on the swollen blind ends of the lymphatic vessels of the synovial tissues.

Dr W. F. Harper read a paper on 'Vital staining of the reticulum and lymphoid tissue of the rabbit appendix'.

Lymphoid follicles form the greater part of the thickness of the wall of the rabbit appendix. Perifollicular lymph channels connect directly with the mucosal lymph channels and the apices of the follicles project into the appendical lumen. Small lymphocytes form the bulk of this lymphoid tissue. A lymphoblastic core is lacking. The central parts of the follicles contain numerous macrophages, moribund cells and cell debris. Following vital staining in moderate concentration, dye inclusions appeared first in cells closely associated with perifollicular and mucosal lymph channels, and many projected into their lumina. Follicular lymphocytes and the central macrophages were not affected, but moribund cells and cell debris were diffusely stained. Higher concentrations of the dye increased the number of stained cells in the perifollicular and mucosal reticulum. Animals killed at the later periods showed appreciable numerical reduction of dye-containing cells at these sites, largely due to a migration of the cells into the follicles, where many were phagocytosed among the central cells. There was evidence of the elimination of the dye into the appendix lumen through the apices of the follicles. Prolonged dye administration increased the number

of stained cells within the follicles, the increase being partly due to the staining of certain follicular cells (possibly lymphocytes) themselves, which had developed in a phagocytic direction as a defence reaction to the increased stimulation. Dye inclusions were also observed in the cytoplasmic processes of the reticulum fibres in intensely stained animals. These appearances are discussed with special reference to macrophage renewal and to the problem of phagocytes in the appendix.

Professor YOFFEY said he would be chary of describing macrophages in the germ centres of the follicles, for the cells in question might be reticulum cells. He would also be chary of describing lymphocytes as taking up trypan blue particles, as it was doubtful if they were phagocytic. Dr HARPER suggested that under intense stimuli the lymphocytes were converted into macrophages. Professor DAVIES suggested that the phagocytozed bacteria might serve as a source of food supply.

Dr L. BLOMFIELD read a paper on 'Intramuscular vascular patterns in man'.

Following previous investigations, which had shown (*inter alia*) that the extent of necrosis in muscles damaged experimentally by gunshot wounds is related to the intramuscular vascular pattern, the patterns characteristic of the limb muscles in man have been studied radiographically. Various types of pattern are described, and their relation to the susceptibility of different muscles to infection discussed.

The PRESIDENT and Professor DAVIES welcomed this paper as a valuable demonstration of the utility of topographical anatomy, and commented on the manner in which the problems of wartime surgery compelled careful reference to the data of gross anatomy for their solution. Professor KIRK described the results of ligature of certain lower limb arteries, ligature of the a. profunda femoris being frequently followed by gangrene. Dr PRITCHARD said the paper demonstrated the greater clinical importance of the intramuscular anastomoses in comparison with the larger, extramuscular inosculations of limb arteries.

NOVEMBER 1945

The Annual General Meeting of the Society for the Session 1945–6 was held in the Anatomy Department, St Thomas's Hospital Medical School, S.E. 1, at 2 p.m. on 30 November 1945, the President (Professor J. C. BRASH) in the Chair.

Dr R. WHEELER HAINES read a paper on 'The movements of the first rib'.

The movements of the first costal ring have usually been described as a simple lid-like raising and lowering of the ring as a whole about a horizontal axis common to both ribs. In fact each rib turns about its own axis, which is so arranged that, as the rib is raised, its lateral border is carried away from the midline, and its pleural surface comes to face less inwards and more downwards. These movements, which can be detected in favourable subjects by palpation, and are easily demonstrable in suitable X-ray films, lead to a much greater expansion of the upper part of the thorax than could occur without them. The peculiar angle made by the neck of the first rib with its shaft, and the atypical articulation of its head with the first thoracic vertebra alone, are necessary for the proper development of its functions.

Professor LE GROS CLARK asked if there was any torsion in the first costal cartilage, and Dr HAINES said that some torsion was necessary to adjust the position of the sternum. Professor BOYD asked what happened when the costal cartilages calcified, and Dr HAINES said that movement was limited, but that the costo-vertebral joints became very lax so that the head of the rib could slide up and down on the first thoracic vertebra. Dr WHILLIS said that there was great individual variability in the movements of the manubrial-sterno joint, and that presumably the more movement there was at this joint the less torsion would be necessary at the costal cartilage.

The PRESIDENT commented on the peculiar movements of the ribs, and Drs FIELD and WEDDELL on the effects that these movements might have in cases of acroparaesthesia and scalenus anterior syndrome.

Dr R. O'RAHILLY read a paper on 'Radial hemimelia and the functional anatomy of the carpus'.

A rare case of multiple congenital anomalies of the hands has been investigated clinically and radiologically. On the right side the thumb is 'floating', that is, it is detached from the skeleton and connected to the hand by soft tissues only. Skiagrams showing the wrist bones of this and other similar cases described in the literature have been examined. The modifications of the carpus thus found are considered to throw emphasis on the trapezium as a constituent of the functional thumb and on the functional subdivision of the carpus described by MacConaill (1941).

Dr J. S. BAXTER read a paper on the 'Effects of pituitary adrenotropic hormone upon cell multiplication, and the distribution of lipoid and birefringent material in the rat adrenal cortex'.

Continued treatment of intact, adult, albino rats with pituitary adrenotropic hormone has yielded the following results.

The hormone does not directly stimulate cell division in the adrenal cortex. Its primary effects appear to be mobilization of sudanophil lipoid and birefringent material (presumably cholesterol esters), loss of these substances taking place from the cortex during the first two weeks of treatment. Later, the amounts of both substances increase again, until the total approaches that in the normal. Their distribution is, however, characteristically altered. A significant rise in cortical mitotic rate is found during the third week of treatment; after this time the rate of cell division diminishes. The increase is preceded by an acceleration in the rate of cell destruction in the zona reticularis, and it is interpreted as a compensatory response of the adrenal cortex so that it may function effectively at a higher level of output of its own secretion.

Attention is also drawn to the circulatory changes in the adrenal cortex which accompany treatment with adrenotropic hormone.

In answer to Professor Boyd who asked how the birefringent material was carried to the medulla, Dr Baxter said that it was mobilized into and carried by the capillaries. He could not say why some of the material appeared outside the capillaries in the medulla, but suggested that there might have been a post mortem shift of the material from the vessels to the cells.

Professor Yoffey questioned whether the association of the cortex and the medulla was really fortuitous. Vogt had suggested a reverse connexion, and Raab had shown that a combination of adrenalin and cortical lipoids was very potent in raising the blood pressure. Dr Field said that in removing the adrenal glands Raab had established an artificial Addison's disease, so that the response to the combination was largely due to the re-establishment of the normal level of corticosterone.

Professor W. E. Le Gros Clark presented 'Further observations on the regeneration of muscle tissue'.

Experiments have been carried out to determine whether complete reconstitution of muscle fibres occurs after simple crushing without interruption of the connective tissue elements of a muscle. Such a reconstitution can take place under favourable circumstances, but in most instances repair of the lesion is very incomplete. In other experiments, portions of gracilis muscle were excised and grafted *in situ* to observe regeneration. The latter proceeds with great rapidity. The newly growing fibres follow the orientation of the original fibres in the graft, apparently guided in a direction determined by the remnants of the original endomysial sheaths.

In reply to the President Professor Le Gros Clark said that after interference with the blood vessels supplying muscles revascularization started within a few days, and that the blood vessels spread down with the fibroblasts. There were two main vessels supplying the tibialis anterior in the rabbit, and if only one of these was cut, so as to interfere with the lower third of the muscle, the vascular supply was re-established in 7 days, but if both were cut this took 14 days. In reply to Dr Fielding he said that the pattern of innervation had not been studied yet, but that the material was suitable for such studies, and for experiments on the effects of tension, immobilization etc.

Dr Richardson and Professor Kirk asked whether both the collagenous and reticular fibres were destroyed following devascularization, and how much of the endomysial stroma and sarcolemma survived. Professor Le Gros Clark replied that in general all cellular elements were lost, and that the reticular fibres and sarcolemma sheaths eventually broke up, leaving only a scaffolding of collagenous material to guide the regenerating fibres, but that the sarcolemma was very persistent, and near the periphery of the lesion it might survive and help to support the muscle fibres.

Drs Field and Bacsich asked if regeneration similar to that seen in the experimental material had been noticed in cases of Volkmann's ischaemic contracture. Professor Le Gros Clark replied that though some regeneration had been seen in these cases, this was always abortive, probably owing to the bulk of the tissues affected. In the case of the very small lesions found in the abdominal musculature in typhoid and pneumonia, probably due to small scattered thromboses, the tissues were usually restored completely, leaving no scars.

Dr Fielding said that the slip of the pectoralis major left behind after amputation of the breast might later increase in size, and Dr Bowden mentioned a small series of biopsies which gave evidence of muscular regeneration. In one a complete infarct followed ligation of the femoral vein, and regeneration was seen at the perifery of the lesion, and in another in a boy of ten in which there was good recovery from a severe Volkmann's contracture, biopsy showed a mass of regenerating muscle fibres.

The President commented on the great interest of the experiments.

Dr C. L. Foster presented 'Some observations upon the cytological development of the parathyroid gland of the mouse'.

A study of the Golgi bodies of the mouse parathyroid gland (together with observations upon the staining reactions and cytoplasmic characteristics of the cells), both during development and at

maturity, suggests an interrelationship between the cell types of the adult gland.

At birth, and for a short time afterwards, the gland consists of embryonic stem cells. During subsequent development, these are followed by dark, transitional and clear types. The mitotic activity of the gland—low in very young and in adult individuals—increases rather sharply to a peak at about weaning time and just prior to the final differentiation, which, accompanied by marked changes in the Golgi body morphology, results in the first appearance of transitional and clear cells.

An attempt is made to relate these findings to physiological activity.

In reply to Dr RICHARDSON, who asked if any material from animals treated with vitamin D had been examined, Dr FOSTER said he had had no opportunity to do so, but that it had been done in the rat. He added that in the rat the administration of parathormone led to the degeneration of the Golgi apparatus.

Professor LE GROS CLARK doubted the value of mitotic counts made on colchicine-treated material as they might be affected by a differential susceptibility to colchicine. Dr FOSTER said that in untreated material there were very few mitoses in young animals and more later, and this agreed with the observations made after the use of colchicine.

Dr H. HUGHES read a paper on 'The regeneration of the islets of Langerhans in the laboratory rat'.

By treatment with appropriate doses of Alloxan a selective necrosis of certain islet cells can be produced. The bearing of the subsequent changes upon the interpretation of certain features of the structure of the normal islet is discussed.

Professor LE GROS CLARK congratulated the author on the excellence of his histological material, and asked if the degenerating cells seen after treatment with alloxan could be stained differentially with trypan blue. Dr HUGHES said that he had some experiments in progress involving the use of intra-vitam stains.

Dr RICHARDSON said that cells intermediate between the acinar and islet types similar to those Dr HUGHES had described in the rat, had been seen in the dog pancreas. The classical work of Bensley suggested, however, that the new formation of islet tissue was from the duct epithelium, not from the acini. Dr RICHARDSON had himself seen occasional cells occupied partly by granules which were apparently zymogenic and partly by beta granules, but thought that the first type were degenerate rather than truly zymogenic, and he doubted the possibility of distinguishing cell types by their inclusions. Possibly the transitional cells were derived from centro-acinar and not from acinar types.

Dr HUGHES said that there was no evidence that the cells were centro-acinar in nature, indeed their diameter corresponded with those of the typical acinar cells, not with the centro-acinar cells which were rather small. He agreed, however, that staining reactions gave slender evidence on which to distinguish cell types.

Professor BOYD asked if the transitional cells had been seen only after alloxan treatment. Dr HUGHES answered that they could be found in about 10% of normal islets, but that they were much more common after the administration of alloxan and were seen at one or two points on the periphery of nearly every islet.

Drs P. BACSICH and G. M. WYBURN read a paper on 'Experimental homografts in guinea-pigs'.

Homografts of cartilage and cornea appear to survive and are used successfully in plastic repair. Homografts of costal cartilage, trachea and oesophagus, cornea, ovary, spleen and a number of other tissues were implanted subcutaneously into guinea-pigs and recovered after an interval of 21 ± days. All homografts were taken from adult animals. With the exception of those of cartilage and cornea, the homografts were either completely replaced by host tissue or were profoundly altered. The significance of these results, confirming those previously obtained by many other workers (particularly Loeb, 1930), is discussed, and a tentative explanation is offered to account for the survival properties of cartilage and cornea.

Dr PRITCHARD said that small pieces of liver, spleen and other tissues would survive transplantation, and Professor LE GROS CLARK suggested that vascular conditions, rather than the presence of muco-proteins as suggested by the authors, were responsible for the survival of homografts transplanted to the brain.

Dr HAINES suggested that the survival of relatively large homografts of cartilage and cornea might be due to their low rate of metabolism, so that their vascular requirements would be less than those of other tissues. Drs BACSICH and WYBURN referred to instances where the tissues near the homograft had become infected, so that the muco-proteins of the graft were destroyed by bacterial products. When this occurred the graft had not survived, and they believed this was due to the removal of the protective muco-proteins.

Professor BOYD drew attention to the survival of homografts of teeth including the dental sacs, and the continued development of the teeth. Here muco-proteins could not be involved.

Drs R. BARNES, P. BACSICH, G. M. WYBURN and A. S. KERR read a paper on 'The fate of nerve homografts in man'.

Of eight cases of human nerve homografts, all failures, three were examined histologically after removal at 140, 355 and 904 days respectively. The points of interest are the partial re-innervation of the grafts, the fascicular necrosis and the subsequent fibrous replacement of the nerve elements of the non-innervated parts of the grafts. Seddon & Young (1942) believe that in the re-innervated homografts of rabbits there is proliferation of the graft Schwann cells and that the invading axons occupy the endoneurial tubes of the graft. Present findings are, however, equally compatible with the conclusions of Bentley & Hill (1936) that the graft acts merely as a scaffold for the regenerating nerve fibres and ultimately is completely replaced by proliferating host fibrous tissue.

The PRESIDENT regretted that Professor YOUNG was unable to take part in the discussion, but welcomed him to the society in his absence.

Professor LE GROS CLARK suggested that genetic factors might play a more important part in the determination of the constitution of the body fluids in man than in the rabbit, and that differences in genetic constitution might account for the failure of human nerve homografts.

FEBRUARY 1946

An Ordinary Meeting of the Society was held in the Anatomy Department, St Mary's Hospital Medical School, from 2.30 to 7.15 p.m. on Friday, 22 February 1946, the President (Professor BRASH) in the Chair.

The following communications were received:

Dr J. J. PRITCHARD: 'Stimulation of new bone production.'

Previously reported experiments showed that the extent of new bone production after fracture of the cranial vault depends upon the severity of the inflammatory reaction in the loose connective tissue above the pericranium. This relationship has been further investigated in the hope of throwing light on the normal stimulus to bone production in fracture repair.

(1) In rats, without bony injury, an acute inflammatory reaction beneath the scalp was induced with turpentine, and the changes occurring in the loose connective tissue were compared with those taking place beneath the periosteum. No bony stimulation occurs during the initial fibrinous and leucocytic exudation, but simultaneously with the appearance of fibroblasts and new capillary sprouts around the inflammatory focus, osteoblasts begin to proliferate beneath the pericranium except (*a*) in the *immediate* vicinity of the exudate, where osteoclasts appear instead, and (*b*) where the m. temporalis with its dense fascia covers the skull. The diploic and dural osteoblasts are not affected.

(2) Closed fractures were produced in the tibiae of rats, and it was observed that new bone first appears beneath the periosteum which lies adjacent to damaged muscle infiltrated with inflammatory exudate. This exudate, unlike that produced by turpentine, was almost free from polymorphonuclear leucocytes. Again, the proliferation of osteoblasts and fibroblasts began simultaneously.

It is concluded that an essential stimulus to new bone production in the early days of fracture repair resides in the inflammatory exudate, after leucocytic emigration has ceased. The poverty of new bone production after cranial fracture may well be due to the absence of concomitant damage to muscles, as this appears to be a major cause of the widespread exudate found after other fractures.

Professor DURWARD suggested that the rate of healing of fractures might be correlated with the blood supply of the region concerned, for in man the lower half of the tibia heals less readily than the upper half where the supply is better. Dr PRITCHARD said that in his material the whole area of the fracture was vascularized in 7–8 days, so that he could not account for his findings in this way.

Professor DE BEER asked whether all areas of the periosteum and dura were equally capable of healing, or whether the suture pattern affected the reaction to injury. Dr PRITCHARD said that healing depended on the activity of the subperiosteal tissues rather than on the fibrous layers of the periosteum, and that once fibrous tissue had been laid down the osteoblasts found it difficult to penetrate the tissue, so that the sutures were preserved unchanged.

In reply to Professor LE GROS CLARK, who asked how far the osteoblastic reaction could be a response to a direct stimulus of injury, Dr PRITCHARD said that the reaction was a distant effect and that direct damage to the osteoblastic layer delayed the reaction. A mere scratching might delay all reaction for as much as ten days.

Dr W. BRANDT: 'Factors influencing the shape of the shoulder joint in the amphibian embryo.'

It has always been believed that the mobile humeral head plays the principal part in fashioning the concave glenoid cavity. The fact that the shape of this cavity is maintained when the upper limb is paralysed has been explained as due to passive movements.

The following experiment was conducted in order to eliminate the possibility of either active or passive factors influencing the shape of the shoulder joint.

In the tail-bud stage of the amphibian embryo (*Amblystoma mexicanum*, stage 35, Harrison), a piece of ectoderm was transplanted from the tail-bud of another embryo into the primordium of the limb. The transplant was attached to the surface where normally the limb developed, and this successfully prevented the outgrowth of the upper limb. The humerus grew within the tissue of the body wall; the elbow joint lay inside the skin. The shoulder joint of this experimentally produced immobile limb showed a normal humeral head and a normal concave glenoid cavity.

It may be concluded from this experiment that the shape of the shoulder joint in the early morphogenetic period of development is not due to the movement of the limb, as has been supposed, but to the pressure of the growing cartilaginous cells of the head of the humerus acting upon the cartilaginous blastema of the scapula. It is the pressure of the primary proliferation centre, the head of the humerus, upon all aggregations of lower mitotic rate in the scapula.

Professor De Beer commented on Braus' classical experiments on the pectoral girdle, and on the differences between Amphibia, where the head of the humerus leads in development, and birds, where the wall of the scapular cavity is formed first. He mentioned Huxley and Murray's demonstration that the isolated femur of a chick can be grown without an acetabulum, and asked if it would be possible to grow the pectoral girdle without a humerus.

Professor MacConaill quoted the case of a man, the head of whose humerus was concave while the glenoid surface was convex, without any associated abnormalities of muscles or disability of function.

Dr Whillis suggested that the poor development of the shoulder joint and the absence of a synovial cavity on the operated side, were due, not to the absence of movement, but to the inhibitory effect of the implanted ectoderm. He added that in the human finger at a stage when there was an apparent cartilaginous union between the articular cartilages the joint was already moving, and that the joint space was filled, not with normal synovial fluid, but with a mass derived from the attrition of cartilage matrix. The shape of the femoral head could be changed in later stages of growth by division of the rotator muscles.

Dr Haines could not accept the suggestion that the pressure of the head of the humerus affected the shape of the scapula, for at the critical stage of development, about 13 mm. in the human embryo, there was a pad of soft tissue, incapable of transmitting pressures, between the cartilages. Hesser had, after a careful study of many joints, discarded all mechanical theories of the causation of the primary shapes of joints.

In reply Dr Brandt said he would attempt to grow a limb-free scapula. He mistrusted deductions from the results of descriptive embryology and hoped to decide doubtful questions by experimental procedure.

Professor M. A. MacConaill: 'The significance of saddle joints.'

Joint surfaces are of two kinds: (*a*) the synclastic, of which the hip and shoulder are species; and (*b*) the anticlastic, of which the saddle joints are species. At any joints, two successive movements about different axes necessarily produce a third displacement (conjunct displacement) about the third principal axis; e.g. lateral rotation of humerus consequent upon flexion followed by backward swing of the arm at the shoulder.

Two displacements at a synclastic joint in, say, a clockwise sense produce a clockwise conjunct displacement (homostrophic rotation). Two clockwise displacements at an anticlastic joint produce an anti-clockwise conjunct displacement (heterostrophic rotation).

The saddle joints are found where unlike conjunct displacements have to be associated with like principal displacements about the other axes at each joint, if the mechanical ends of the systems considered are to be achieved.

Dr Haines suggested that though saddle joints were rare in man they were common in fishes, and that a comparative study might lead to a better understanding of their significance. They could be compared with the region of contact between two links of a chain, following Du Bois-Reymond. Dr Haines demonstrated a model built up on such links, and claimed that the known properties of saddle joints were demonstrated by this model, particularly the limited amount of rotation found at such joints.

Professor MacConaill said that he too had begun with such crude models, but that joints could not be understood without a critical apparatus of geometrical principles. The carpo-metacarpal joint of the thumb and the wrist joint had similar ranges of movement, but were different in construction.

Drs P. Bacsich and G. M. Wyburn: 'Effect of combined chorionic gonadotropic and anterior pituitary hormones on the immature female guinea-pig.'

Previous work (Bacsich & Wyburn, 1942) has shown that full uterine heat response (including antimesometrial hyperaemia) cannot be elicited in the immature female guinea-pig with either direct

or indirect (Antostab—Boots, Ltd.) oestrogenic stimulation.

Experimental and clinical evidence suggests qualitative and quantitative improvements in the ovarian, and probably also in the uterine, response in immature animals after the administration of combined chorionic gonadotropic and anterior pituitary hormones.

On the basis of the above evidence, combined chorionic gonadotropic and anterior pituitary hormones (Synapoidin—Parke, Davis & Co.) were administered to immature female guinea pigs in repeated subcutaneous injections. Results will be described, discussed and compared with findings of earlier experiments and with statements in the literature.

Dr PRITCHARD said that in the rat as in the guinea-pig lutein cells were derived from the theca interna.

Dr R. J. HARRISON commented on the small effect Synapoidin had when administered to human beings.

Dr E. W. WALLS: 'Development of the specialized conducting tissue in the human heart.'

The several parts of the cardiac conducting system, bundle and limbs and both nodes, have been identified in specimens as small as 16·5 mm. The early definition of the SA node described by Sanabris has been confirmed. An interesting feature is the much greater relative size of the bundle in the pre-natal heart. Attempts have been made to correlate foetal electrocardiographic tracings with developmental structural changes.

Professor MACCONAILL congratulated Dr WALLS on his success in demonstrating human Purkinje tissue.

In reply to Dr FIELD, Dr WALLS said that he had found the continuity between the muscle of the auricle and ventricle broken at about 11 mm., whereas he had not been able to recognize the bundle till the 16 mm. stage, certainly not at 8 mm., the stage at which Tandler had described it. This left the problem of the transmission of the stimulus for contraction from the auricle to the ventricle unsolved.

Dr BACSICH advocated the use of large blocks rather than small blocks for the demonstration of conducting tissue.

Professors KIRK and BOYD and Dr FIELD discussed the terminology of the auriculo-ventricular cushions, and Professor BOYD's suggestion that they should be distinguished as mesocardiac and anti-mesocardiac was generally accepted.

Dr FIELD said that in the sheep embryo no cushions were developed when the heart was in the tubular stage, though they appeared later.

Dr A. E. BARCLAY showed on behalf of Professor H. J. SEDDON a cineradiographic film of 'Human joint movements'.

Professor MACCONAILL commented on the excellence of the film and the clarity with which it showed the velocity gradients of the moving parts.

Dr F. P. REAGAN: 'The later development of the right atrium in certain quadrupeds.'

A great length of cardio-vascular structure, usually called thoracic postcava in quadrupeds, as compared with that of thoracic inferior cava in man, was noted by early observers and correlated with development of the infra-cardial lobe of the lung.

Attenuation is commonly said to be a feature of the thoracic postcava proper of quadrupeds: the possibility that the sinus venarum of the right atrium shares in this attenuation has not been completely explored embryologically.

Auricular appendages are often labelled 'atria' by those who maintain that foetal atria cannot communicate.

Sinus valves are formed at the junction of sinus venosus and primitive atrium from which the left atrium is budded. Attenuation of the caudal part of the residue of primitive atrium would result in great caudal displacement of the sinus valves: attenuation of the sinus venosus might result in a minimal displacement. A study of a closely graded series of ontogenies in which well-developed sinus valves become caudally shifted is epitomized, and the role of the left sinus valve in the formation of so-called tubercle of Lower is discussed.

Dr FIELD said that he had found sheep embryos of 18–19 days very variable in their development, particularly as regards the development of the left sinus valve.

Dr G. M. WEDDELL: 'Anatomical considerations in the treatment of traumatic epistaxis.'

When cases of epistaxis do not respond to local measures, the usual surgical practice is to ligate first the external carotid artery and, if this is not successful, the internal carotid artery, in order to control the haemorrhage. These surgical methods are not always successful and, despite their application, cases of death from epistaxis have been reported. In this communication six cases of epistaxis are described which were not controlled until the ethmoidal arteries on the affected side had been occluded. The anatomical approach in the surgical treatment of traumatic epistaxis is discussed.

Dr D. A. W. EDWARDS (introduced by Professor H. A. HARRIS): 'Blood supply and lymphatic drainage of tendons.'

The distribution of arteries, veins and lymphatics

in tendons and tendon sheaths has been studied by India ink and vermilion cream injections in man and various animals.

The specific pattern of the vessels in the tendon, musculo-tendinous junction and osteo-tendinous junction is given. The nutritional features of the tendon and tendon sheath are discussed in relation to repair.

Dr D. V. Davies: 'Some observations on the anatomy of *Nycticebus*.'

Apart from the blood-vascular bundles in the limbs and the curious coiling of the colon, the slow loris presents several other interesting features. These include (*a*) unusual arrangement of the valves in the right atrium; (*b*) a high incidence of double inferior vena cava; (*c*) valves in the portal vein; (*d*) an os clitoridis; (*e*) sesamoid bones in the extensor tendon of both manus and pes.

Dr Haines referred to the recent report of the presence of sesamoid bones in the extensor tendons of the mole, where they had been ascribed to pressure of the soil on the back of the hand in burrowing, and asked whether there was any similar habit to account for their presence in *Nycticebus*, or if they were associated with maintenance of the blood supply in these tendons when they were stretched over the flexed joints.

Professor Osman Hill said that *Nycticebus* grasped branches for long periods and no doubt the sesamoids in the tendons and the retia mirabilia along the course of the limb vessels were associated with this habit.

Professor Cave said that, in the larger Canidae, at least, dorsal sesamoids occur on the extensor aspect of the metapodiophalangeal joints. These bones are smaller than their flexor counterparts and are generally lost from the skeleton during maceration. An intensive search of a sufficiency of carefully prepared mammalian material would almost certainly demonstrate the occurrence of these extensor sesamoids to be much more common than at present supposed.

Dr Reagan asked for an account of the aortic sympathetic plexus.

Dr G. J. Romanes: 'Chromatolysis in developing nerve cells.'

In the course of mammalian development many groups of nerve cells undergo changes indistinguishable from the chromatolysis of axon injury. This phase affects almost all the cells of any one nucleus at the same time, and in many cases coincides with the development of myelin in their axons. The presence of such a developmental stage is further evidence of the physiological nature of this histological picture.

In reply to Professor Zuckerman, Dr Romanes said that in the inferior olive 70–80 % of the cells might show gross chromatolysis, and the other cells be more slightly affected, but in later stages no such extensive changes could be found.

Dr Romanes agreed with Professor Young's suggestion that the chromatolysis might be associated with the sudden increase in diameter of the fibres at this stage, resulting in a drain on the cell body.

In answer to Professor Boyd he said that in sheep embryos he had seen chromatolysis of the red nucleus, anterior horn cells, Clarke's column and other nuclei, in each case associated with nerve tracts about to become medullated.

Professor W. C. Osman Hill: 'Cauda humana in a Tamil.'

Comprehensive studies by Bartels (1880, 1884) on the frequency, structure and geographical distribution of human tails and tail-like appendages leave little scope for additional data. But the example now reported pertains to a race and region not represented in Bartel's series and is, moreover, difficult to classify in his scheme.

An Indian Tamil boy (aet. 11, stature 1164 mm.) of coolie class, observed in Colombo, Ceylon, possessed a tail-like appendage (150 mm. long and 45 mm. thick at its base) springing from the midline of the back at the level of the iliac crests. It was twisted (1½ coils) and was applied to the right side of the back, the coils proceeding clockwise, resembling the tail of a pig. Passive manipulation permitted the appendage to be extended, but thereafter it returned to the original position. The 'tail' was covered by smooth, hairless skin like that of the back, and was subject to normal cutaneous sensibility. Internally no bony or cartilaginous support could be felt, the core apparently being composed of firm (? connective) tissue with a central fibrous strand. Surgical removal was out of the question, as the parents realized the financial value of the abnormality for public exhibition!

The appendage was no true tail as it was not in line with the coccyx, nor did it contain any vertebral rudiment. It was, therefore, some type of cauda spuria, falling best into Bartel's category of 'tail-like structures in anomalous sites'. It was probably not a cystosarcoma, which Bartels avers to be the commonest in this position, but may have been a form of sacro-coccygeal tumour, though its shape seems to rule out any parasitic twin; it may, on the other hand, have been an effect of spina bifida.

A similar appendage is quoted by Bartels from Roloff (1761), but this was not median. The Moi (Indo-China) boy reported by Wiedersheim (1895) is probably in the same category.

Professor W. C. Osman Hill: 'Albinism in man, etc., in Ceylon.'

Albinism is specially prevalent in Ceylon. Personal observation has established its occurrence in man, monkeys, shrews, palm-squirrels, bandicoots, deer, crows, mynahs, peafowl, cobras and kraits. In the majority, including man and the macaque monkey, albinism is complete.

In man albinism was observed in three scattered families of Sinhalese and in one Tamil family. Only in the last mentioned was close investigation possible. Here two children, 1 ♂, 1 ♀, out of a family of four, were albinos; parents were both normal, but a maternal aunt was albinistic. No instance of piebalding ('magpie-nigger') was seen in man, but isolated instances of white forelock were seen in several districts. All were in Sinhalese, and both sexes were affected.

In contrast, albinism in the leaf-monkey (*Kasi senex*) is invariably of the piebald type, with pigmented face and eyes, piebald hands and feet and patchily pigmented pelage. Like true albinism, the condition is inherited.

Albinism is commonly accepted as due to gene-mutation, and is known to behave as a Mendelian recessive. Its frequency in so many individuals of so large a variety of species in Ceylon therefore raises a difficult problem. If, as seems likely, some factor or factors, involving geological and/or climatic features, is affecting the fauna, the possibility of environmental impress on germ-plasm must be postulated. Otherwise 'mere coincidence' must be relied on as an unconvincing 'explanation'.

Dr R. G. Harrison: 'Relation of the adrenal cortex to structural changes in the heart and kidney.'

Selye *et al.* (*J. Amer. med. Ass.* 1944, **124**, 201) claim to have produced myocardial Aschoff bodies and nephrosclerosis by repeated injections of desoxycorticosterone acetate in rats. Repetition of these experiments has shown that such structural changes are the result of infection and not desoxycorticosterone acetate, and that the latter may be actually concerned in the resistance of the organism against such lesions.

Dr R. J. Harrison: 'Early development of the corpus luteum in the mare.'

By rectal palpation of the mare's ovaries it is possible to estimate the time of ovulation to within 6 hr. By this method a series of corpora lutea were obtained from mares killed 18 hr. to 14 days after ovulation. A further series was examined, in which the largest follicle was punctured through the anterior vaginal wall just before ovulation was thought imminent by rectal palpation. The mare was then killed a known time after the follicle had been ruptured.

The two series are compared histologically and the fate of the layers of the follicular wall is discussed.

JUNE 1946

The Summer meeting of the Society was held in the Department of Anatomy, University College, Cardiff, on Friday and Saturday, 28 and 29 June, the President (Professor Brash) in the Chair.

The following Communications were received:

W. E. Le Gros Clark and M. Meyer: 'Study of the connexions of the olfactory bulb by terminal degeneration.'

Within a few days after a lesion in the olfactory bulb of the rabbit, characteristic changes occur in the terminal fibres of the olfactory tract, as seen in silver preparations. These changes are shown in enlarged and deeply staining boutons and swollen and beaded terminal aborizations. As the result of several experiments, such terminal degeneration has been seen in the opposite bulb, the pre-piriform cortex, the plexiform layer of the piriform cortex, the nucleus of the lateral olfactory tract, the central and basal elements of the amygdaloid nucleus, and the olfactory tubercle. The last is of particular interest, since the direct connexion of the olfactory bulb with the olfactory tubercle has been questioned by some authorities. No terminal degeneration could be found in the septal nuclei, the nucleus accumbens, the diagonal band of Broca or the dentate gyrus.

W. E. Le Gros Clark and R. T. T. Warwick: 'The pattern of olfactory innervation.'

As part of a general investigation of the olfactory system, the mode of distribution of the olfactory nerves and their termination in the bulb have been studied in the rabbit. Contrary to the usual diagrams, no plexiform arrangement of olfactory fasciculi occurs in the nasal mucosa. The olfactory nerves pass upwards to the cribriform plate in separate parallel bundles. Above the cribriform plate, however, a most intricate plexus occurs in which the olfactory fibres undergo complete rearrangement. If the olfactory bulb of a rabbit is removed, the olfactory epithelium degenerates with remarkable rapidity, and all the sensory cells practically disappear within a week. If a portion only of the bulb is removed, there is not a total atrophy confined to a corresponding part of the

mucosa; on the contrary, there appears to be a general thinning out of the olfactory cells over all the mucosa. Thus it appears probable that each local area of the mucosa is projected on to every part of the bulb. Such a 'sorting out' of the olfactory fibres would be consistent with the conception that the olfactory epithelial cells are specific types, serving as so many peripheral analysers in the function of olfactory discrimination.

The above two communications were taken together.

The PRESIDENT and Professor YOFFEY asked whether there was evidence of functional differentiation, either in the structure of the sensory cells of the olfactory epithelium, or in the way in which the fibres from these cells spread out on reaching the olfactory bulb. Professor LE GROS CLARK said the terminal swellings of the peripheral processes of the olfactory cells (olfactory rods) and the olfactory hairs showed certain morphological variations in the sections which had been prepared, but it was not possible yet to state whether these were normal or constant variations. More histological material was required, particularly in view of the difficulty of staining these structures. The rearrangement of the fibres as they reached the bulb did suggest a peripheral analysing mechanism.

Dr OSMAN HILL enquired about the argentophil granules in the layer of mucus which were seen in some of the sections. Professor LE GROS CLARK said that the granules, which often appeared in rows, were perhaps attached along the length of very fine terminal hairs, though these hairs were themselves sometimes invisible. The granules might be secretion products of Bowman's glands, rather than foreign particles. It was believed on good evidence that substances had to be soluble in both water and lipoids before they could be smelt, for complete insolubility in either prevented their detection.

Professor BOYD commented on the fact that in the embryo the olfactory bulbs were induced by the olfactory epithelium, yet in the adult removal of the bulbs led to the complete disappearance of the sensory cells of the epithelium by retrograde degeneration. Dr ROMANES spoke of retrograde degeneration in new-born mice, where destruction of the anterior roots of the spinal nerves led to the complete atrophy of the anterior horn cells within a week, and there was no sign of regeneration later.

Dr PRITCHARD suggested that if the impulses arising as the result of different stimuli were distributed to different regions of the bulb it should be possible to distinguish between the local electrical reactions to these stimuli. Professor LE GROS CLARK said that they had the possibility in mind, and that it would be worth trying out in a big animal.

Dr HAMILTON commented on the complete absence of fibres shown in lantern slides of the prepiriform cortex after destruction of the bulb. Professor LE GROS CLARK said that the sections had been taken through the plexiform layer of the cortex, and that in this region, since all the fibres arose as collaterals from the olfactory tract, they all disappeared.

P. GLEES and P. WALL: 'Fibre connexions of the subthalamic region.'

Experimental lesions were made in the subthalamic region of the brain in five macaque monkeys, and the resulting degeneration was studied by the Marchi technique.

The subthalamic nucleus is shown to project on to the external and internal division of the globus pallidus and on to the red nucleus of the same, and of the opposite, side. Further fibre contributions are made to the thalamus and to a nucleus situated medial to the substantia nigra. This last sends fibres into the globus pallidus, to the centre median nucleus and to the anterior colliculus. The fasciculus longitudinalis dorsalis of Schütz receives fibres from the subthalamic region.

Professor LE GROS CLARK said that in this region, where the fibre systems were diffuse, it was difficult to limit experimental lesions, and the connexions of the nuclei were only gradually to be disentangled. In the opinion of many, the Marchi technique gave rather doubtful results which required to be confirmed by other methods. He questioned the existence of the connexions between the ventral nucleus of the thalamus and globus pallidus described by Dr GLEES, for if all the cortex were removed all the cells of the ventral nucleus degenerated, and none remained which might give origin to fibres going elsewhere. The question whether the centre median nucleus was a thalamic element or not was really one of definition; it was certainly thalamic in topography and embryology. Dr GLEES answered that the fibres he had described as going to the globus pallidus were perhaps not so much direct fibres as collaterals, and that after the destruction of the important connexions of a nerve cell the body might disappear in spite of the existence of such collaterals, so that the experiments mentioned by Professor LE GROS CLARK could not be taken as evidence against their existence. The ventral nucleus of the thalamus was mainly a relay centre concerned with the transmission of the impulses to the cortex, but collaterals might go elsewhere. Professor LE GROS CLARK remarked that in the cortex cells might persist after destruction of their main fibres and this was probably due to the existence of collaterals. If the collaterals going to the globus pallidus existed it should be possible to demonstrate them by the use of a silver technique.

Dr HAMILTON commented on the clarity of the dorsal longitudinal bundle at the level of the inferior colliculus and its apparent absence opposite the superior colliculus, though Dr GLEES had described its fibres as rising higher still in the subthalamic region. Dr GLEES said that long descending fibres of the bundle could be followed from the subthalamic region by the degeneration technique, and that these were present at the level of the superior colliculus, but were less concentrated at that level than lower down. Running with the long fibres were shorter fibres arising in the dorsal tegmental substance.

J. M. YOFFEY and J. S. BAXTER: 'The formation of birefringent crystals in the suprarenal cortex.'

It has been known for many years that birefringent crystals occur in the suprarenal cortex. Since crystals of desoxycorticosterone acetate (and presumably other corticosterones) are also birefringent, it seemed worth while investigating the distribution of these crystals more closely, for the light they might possibly throw on cortical secretion. In freshly cut frozen sections of the rat suprarenal there are no birefringent crystals, either acicular or spherical. If the unfixed sections are mounted in normal saline, distilled water, or syrup of laevulose, as well as a number of other media, and allowed to stand for some days, crystals gradually form—partly in the zona glomerulosa, mostly in the outer part of the zona fasciculata, and to a lesser extent in the inner fasciculata and the reticularis. Occasional spherical crystals, with the typical black cross of polarization, occur in the inner part of the zona fasciculata. No birefringent crystals occur in the medulla.

The same phenomenon may be observed in the suprarenal gland of the cat and the horse. In the cat there is a very plentiful formation of crystals in Bennett's secretory zone. The crystals are possibly formed by the break-down of a more complex molecule such as a lipoprotein.

Dr GLEES asked how differences of *p*H and temperature affected the formation of the crystals. Professor YOFFEY said he could as yet make no definite statement on this point, but that he was trying out the effect of *p*H changes. He added that possibly the birefringent material was combined as a lipoprotein in the tissue, and that this was gradually broken down so that the material eventually crystallized out. Treatment with pepsin, however, did not lead to a more rapid formation of the crystals, so there was no direct evidence for this hypothesis.

Professor BOYD suggested feeding cholesterol to experimental animals to see whether the crystals were increased in amount. Professor YOFFEY said that he had considered this procedure, but had been unable to obtain cholesterol for the purpose. He had tried the effect of a number of fat solvents on his sections, but had found no solvent by which he could differentiate between fats and sterols. He agreed with Dr PRITCHARD that the birefringent material might be in solution in the fat of the fresh tissue, and crystallize out after fixation of the tissue. In the guinea-pig liver there could be 3% or more of free cholesterol dissolved in the fat without the appearance of birefringence. Lipoproteins made up a quarter of the total plasma proteins, yet there were never crystals in the capillaries.

Dr BACSICH asked about the fixation of the material, as anomalous staining of Weigert sections of the brain was sometimes due to lipolysis. Professor YOFFEY answered that the tissues were fixed for a minimum of 24 hr. in formalin. He added that sometimes fats might crystallize out after formol fixation.

J. B. ROSTON and R. WHEELER HAINES: 'Joint "cracks".'

When the bones articulating at a joint are pulled apart, the articular surfaces at first separate slightly, giving in favourable subjects an increase of the articular gap, as measured in radiographs, of about 0·5 mm., and a sensible and audible knock when the articular surfaces are pushed sharply into apposition. This phase of initial separation ends suddenly as the tension is increased, with a further separation of the articulation elements by about 2 mm., the production of an audible 'crack' or 'snap', and the associated appearance of a 'bubble' visible in the synovial fluid in the radiograph. This bubble is presumably filled with water vapour and blood gases under reduced tension, and once formed it can be maintained or enlarged by moderate tension, less than that required to form it initially. If tension is relaxed the bubble is dissolved, but the persistence of the bubble for about 20 min. is presumably responsible for the difficulty in cracking the joint a second time by simple tension.

There is great individual variation in the ease with which cracks are elicited. The best cracks at the metacarpo-phalangeal joint are heard when the articular elements spring apart at about 7 kg. tension, and, taking into consideration the restricted surface area of these joints, such a tension must indicate an absolute negative pressure of some atmospheres. Exhibition of the intrinsic tensile strength of fluids is rare in laboratory work, as most fluids contain gas nuclei about which bubbles are formed easily at low pressure, but in biological systems where the walls of the vessels are perfectly wetted the fluids may be able to sustain considerable absolute negative pressures, and the cohesive

strength of a joint due to this property may be far beyond what could be expected if it depended on atmospheric pressure alone.

In reply to Dr WHILLIS, Dr HAINES said that a depression of the skin over the joint region was seen in favourable cases in the phase of preliminary separation and that it was always seen after the crack, when the bones were well separated and the walls of the capsules sucked in by the strong negative pressure.

Dr PRITCHARD suggested that the crack might be due to the breaking apart of two articular surfaces cohering by a thin layer of synovial fluid. Dr HAINES said that this might account for the noise, since two glass slides held under water and suddenly pulled apart could give an audible crack. It was, however, possible to demonstrate a considerable separation of the joint surfaces before the crack by palpation, by knocking when the bones were forced together, usually by denting of the skin and by radiographic methods, so that the suggestion had been set aside.

J. WHILLIS: 'Synovia in developing joints.'

Free movement of the limbs occurs in newts at the stage when there is apparent cartilaginous continuity between the joint elements. Similar observations were made by R. W. HAINES on the joints of a young kitten. Artificial shrinkage of the cartilage demonstrates that the surfaces, although separable, are not uniformly smooth. It is suggested that attrition of the cartilage makes a large contribution to the synovia at this stage, and that, on fixation, the fluid matrix is similar in appearance to the normal matrix.

Dr HAINES showed a series of slides illustrating the development of the synovial cavities in man. He said that all joints passed through a stage at which the interzones were three-layered, with two outer dense layers that eventually formed the articular cartilages, and an intermediate loose layer that broke down to form part of the synovial cavity between the cartilages. He believed that once the cavities had been formed, at about 30 mm. in the larger joints, they persisted throughout later development, but their presence was often obscured in histological preparations by adhesions due to artefacts. In man he had found no evidence of attrition of the cartilage in embryonic life.

In reply to Dr BACSICH, Dr WHILLIS said that in sections stained with toluidin there was no difference in the metachromatic staining between the cartilage matrix and the synovia.

R. E. M. BOWDEN and E. GUTMANN: 'Changes in human voluntary muscles in vascular lesions.'

Muscles biopsies were examined from 16 cases with vascular damage.

Contractures had occurred in all affected muscles, but the histological changes showed three main types of lesion. In the first group the predominant change was of necrosis of the muscle with surrounding areas of fibrous tissue proliferation; in the second there was dense interstitial fibrosis; and in the third there was patchy necrosis and fibrosis.

The fate of the muscles depends upon the extent of the damage, the possibility of regeneration of the muscle fibres and the state of the nerves.

The correlation of histological and clinical observations indicates the possible mechanisms underlying the formation of contractures.

Professor LE GROS CLARK remarked that the biopsy method was most promising and needed intensification. Muscle regeneration in rabbits seemed good, but it had to be remembered that in this animal the tibialis anterior only weighed 2 grams, and a similar amount of regeneration in a human muscle would not be clinically significant. He inquired whether nuclear proliferation ('nucleosis') had been seen in the damaged muscle, as amitotic division of the nuclei leading to an arrangement in rows had been described. Dr BOWDEN said that in patients whose biopsies showed evidence of good muscular regeneration, power was eventually regained though the contractures in the muscles persisted. It was difficult, however, to know how much of the clinical restoration of power was due to the regeneration of damaged fibres or to the hypertrophy of the remainder. 'Nucleosis' had been described in fibres which were wavy in outline and showed strong longitudinal striation, and these were marks of denervation rather than regeneration.

Dr WALLS asked whether there was any regeneration in the muscular tissue of the heart after coronary thrombosis. Professor LE GROS CLARK said that after alcholic damage there was no attempt at regeneration similar to that found in voluntary muscle.

D. V. DAVIES: 'Synovial joints in the skate.'

The characters of the large synovial joints of the skate are described. The distribution, structure, development and possible function of intra-articular structures are discussed.

Dr HARRISON remarked that the loose rounded bodies Dr DAVIES had described as occurring in great abundance in the joints of the skate were reminiscent of ball-bearings, and asked if they grew with age, and Dr DAVIES answered that they were undoubtedly larger in older specimens. In answer to the PRESIDENT, who asked if the laminated structure observable in the sections of some of these bodies could be correlated with age, Dr DAVIES said he had no data bearing on the ages of his material.

Dr PRITCHARD asked how the structure of the loose bodies compared with those sometimes found

in human joints, and Dr DAVIES said that in man the structure was not so simple, for they might be fibrous, cartilaginous or bony, and these tissues might be alive or dead. Dr BACSICH suggested that the bodies were synovial rather than cartilaginous in nature. In answer to Professor CAVE, Dr DAVIES said that there was no suggestion of pathological changes in the joints he had described.

J. GUNN ROBERTS: 'Congenital absence of ventro-lateral abdominal musculature.'

The case history of a male child, age 3 months, with congenital absence of the external and internal oblique and transverse abdominis muscles on both sides of the abdominal wall is presented. Both recti were intact and no obvious enlargement of the urinary bladder was evident.

The current aetiological view that the condition is secondary to continued pressure by a dilated bladder in utero is criticized.

In answer to Professor HAMILTON, Dr ROBERTS said that a barium meal had been given to confirm the existence of a suspected diaphragmatic hernia but the radiographs were normal.

Professor WEST said that he had seen Dr ROBERTS' case and that the viscera could be distinguished with extraordinary clarity through the thin abdominal wall where the muscles were absent, but that the rectus muscles were present in their usual position. The case suggested that the rectus had an embryological origin which differed from that of the other muscles of the wall. Professor BOYD agreed with this suggestion, and added that the wrinkling of the skin described by Dr ROBERTS reminded him of a similar case in which a congenital cyst had distended the abdomen and had then disappeared.

Dr OSMAN HILL said that he had dissected two cadavers showing similar defects, but that in his examples the lesions had been unilateral. He regarded the effect as a widening of the linea semilunaris rather than a congenital absence of the musculature, for he had found the muscles well developed, though they were confined to the more posterior parts of the wall.

Dr WALLS remarked that the testicles were undescended, and a study of Dr ROBERTS' case might help to solve the problem of the mechanism of descent.

A. G. H. MITCHINSON and J. M. YOFFEY: 'Respiratory displacement of larynx, hyoid bone and tongue.'

Arising out of a consideration of the degree of mobility of the lung root, attention was directed to the question of respiratory displacement of the larynx. X-rays were taken of twenty-three subjects in extremes of respiration. In fourteen cases there was no vertical displacement of the larynx whatever; in five the larynx rose an average of $\frac{1}{4}$ in. during inspiration; and in four it showed depression of about $\frac{1}{4}$ in. on inspiration. The larynx, in other words, does not undergo appreciable vertical displacement on deep breathing, but this immobility is not passive. On the contrary, there is active contraction of the suprahyoid muscles on deep inspiration, countering, presumably, the downward pull of the trachea.

An unexpected result was a marked forward displacement of the tongue and hyoid bone which was clearly evident in eighteen out of the twenty-three cases. This hyo-lingual displacement no doubt facilitates the ingress of air into the respiratory passages in deep inspiration.

Professor KIRK remarked on the increase of the distance between the hyoid and arytenoid in inspiration, amounting to at least a third of its expiratory value and Dr WHILLIS remarked on the displacements of the hyoid. Professor YOFFEY agreed that these and other changes such as rotation of the cricoid took place during respiration, but said he had avoided mention of them as he wished to concentrate on the special points he had discussed. He was, however, engaged on further work bearing on the subject.

Mr TURNER WARWICK said that if the lower end of the trachea was displaced downwards during inspiration by an amount which stretched it to the limits of its elasticity, it was difficult to understand how the larynx could rise in this phase of the respiratory cycle.

P. BACSICH: 'Multinuclear ova and multiovular follicles in the young human ovary.'

In a recent examination of serial sections of human new-born and infant ovaries, multinuclear ova and multiovular follicles were found with great regularity. Earlier investigators thought their occurrence to be comparatively rare in man and believed that they might furnish explanation of twinning and teratological abnormalities. More recently, Hartman (1926) advanced the view that they represent atretic phenomena. This latter view is accepted, and it is believed that this condition is comparable with the parthenogenetic changes observed (Bacsich & Wyburn, 1945) in the atretic follicles of rodent ovaries.

While other factors may play a role in the production of the above changes, it is suggested that their relative frequency in the young ovary is due mainly to the post-natal withdrawal of gonadotropic hormonal influences.

Dr PRITCHARD said that in a prepubertal rhesus monkey he had found multinuclear ovules in practically all the follicles. Dr BACSICH answered

that he had examined animals 5–6 years of age, and had had difficulty in finding even one or two. He believed that their presence depended on hormonal influence. Earlier workers had had difficulty in obtaining adequate material drawn from a single age group, and this accounted for discrepancies in their estimates of frequency of multinuclear follicles.

Professor BOYD inquired as to the conditions in the ovaries during the 7th–9th month of pregnancy, when placental hormones were available. Dr BACSICH answered that it was generally maintained that these hormones had no effect whatever except on the interstitial tissues.

Professor HAMILTON said he had noticed multiple ova in older women, and had believed that they were the result of the fertilization of normal ova by several sperms. He had come to doubt this explanation and now thought that the presence of three or four pronuclei might be due to the fertilization of abnormal eggs.

W. J. HAMILTON: 'Hydatiform degeneration in an early human embryo.'

The specimen was obtained as the result of abortion. The history of the case is as follows: Last menstrual period on 5 April; Friedman test positive on 7 May; there was continuous loss of blood from 9 to 23 May, probably an abortion; 17 May, Friedman test negative; 19 June, Friedman test again positive; 20 June, abortion of a chorionic sac. One side of the chorionic sac is damaged. On the opposite side a complete yolk sac is found. The amnion is absent. Mesoderm lines the inside of the chorionic sac and the yolk sac, and is found also in the villi. The loose mesenchymal stroma of the villi shows many intercellular spaces. The mesodermal core of the swollen villi gives a metachromatic staining reaction with toluidin blue, indicating mucoid degeneration.

Professor WEST commented on the thickness on the mesoderm of the specimen, and Professor BOYD asked whether the absence of vascularization could be taken as evidence of the absence of the yolk sac and whether the cavity that appeared in the embryo could be interpreted as an exocoelome. Professor HAMILTON answered that if Hertig's view that vascularization took place in the villi was correct the absence of vascularization in the specimen was without significance, and that the cavity was not in the right position for an exocoelome. He agreed with Professor BOYD that the mass of cells which appeared in the roof of the yolk sac and which he had suggested was the atlanto-enteric diverticulum might be the prochordal plate.

In answer to Dr BRANDT, Professor HAMILTON said that the wall of the yolk sac was bilaminar in structure, with a lining of endoderm and a covering of mesoderm. In answer to the PRESIDENT he said he had described the embryo in some detail as it represented a very early stage in the development of the pathological condition.

I. W. MONIE: 'Development of the trigone of the bladder and the termination of the mesonephric ducts.'

The developing bladder region of embryos ranging from 4·5 to 42 mm. has been examined, and the following points noted:

(1) The trigonal region does not appear to receive any epithelial contribution from either the mesonephric ducts or the ureters, and is therefore not of mesodermal origin. In the earlier embryos the dorsal wall of the bladder shows marked cellular proliferation, and cells from this region invade the upper part of the urethra and the terminal portions of the mesonephric ducts. The trigonal area is considered, therefore, to have an endodermal origin.

(2) The openings of the mesonephric ducts and ureters into the bladder become widely separated by an area of relatively rapid growth in the dorsal wall of the bladder. The description by Frazer (1935) of a loop-like formation of the mesonephric duct and the later absorption of the ventral part of this loop into the bladder wall could not be confirmed.

Dr BRANDT said that Frazer had described and figured the loop of the mesonephric duct in most convincing detail, and suggested that the absence of the loop in Dr MONIE's specimens was due to individual variations. Professor BOYD said he had always been suspicious of the supposed mesodermal origin of the trigone but he could not understand the mechanism of its development by proliferation of the endoderm described by Dr MONIE. He had seen proliferation in the region of the Mullerian tubercle, but questioned whether it stretched far enough cranially to account for the separation of the ureteric and mesonephric ducts. At 100 mm. there was no distinction whatever between the epithelium of the trigone and that of the surrounding area, and this might suggest that the mesodermal contribution had disappeared. Dr MONIE answered that cells from this proliferating mass did extend much more cranially than the Mullerian tubercle into the trigonal region. These endodermal cells separated the openings of the mesonephric ducts and ureters and even replaced the mesodermal epithelial cells of these structures in the regions of their mouths. Dr BAXTER said that it was difficult to determine the extent of the epithelia, but that if replacement occurred it should take place on the deep aspect of the epithelial coat. Dr PRITCHARD suggested that it was a question of demarcation of epithelia rather than replacement of one by another.

In answer to Dr ROMANES, who remarked that the distribution of the response to oestrogens

suggested a mesodermal element in the urethra of the adult, Dr MONIE questioned the suggestion that oestrogens should have a specific action on mesodermal tissue. He drew attention to the work of Zuckermann who, by injecting oestrogens into female monkeys, caused a proliferation of endodermal cells in the urethra and vagina which extended cranially into the trigonal region. Burns appears to have had similar results by injecting oestrogens into opossum embryos. Professor HAMILTON said that the ectoderm also reacts to oestrogenic stimulation so that the results were difficult to interpret, and he discussed changes of the cell types in the genital tracts of adult females in pathological conditions. Professor KIRK remarked that each of the three germ layers can give rise to any type of epithelium, so that the structure of an epithelium is no criterion of its origin.

R. J. HARRISON: 'A case of conjoined female twins.'

The case to be presented is that of conjoined female twins obtained by Caesarian section at the end of the seventh lunar month. The twins are joined by ventro-lateral union from the chin to the umbilicus (*Prosothoracopagus* Schwalbe).

Dissection of the thoracic cavity shows a number of abnormalities in the disposition and arrangement of the viscera. The structure of the inverted compound heart and the abnormal arrangement of the pulmonary trunks are described.

In answer to the PRESIDENT, Dr HARRISON said that the presence of the monster had been diagnosed at the sixth month of pregnancy, that it had been delivered by Caesarian section and had lived 5 sec.

Dr BRANDT remarked that it was the first case of its kind with a single heart. In Amphibia the heart primordium could subdivide to form 2–7 hearts, but fusion of separate primordia did not occur. Possibly in man the opposite might take place and two separate primordia fuse. Dr HARRISON said that he had collected records of three similar cases, but without compound hearts.

W. R. M. MORTON: 'Arhinencephaly and multiple developmental anomalies occurring in a full-term human foetus.'

Complete absence of olfactory nerves, bulbs and tracts, bilateral colobomata of the iris, and defective ossification of the parietals are the main abnormalities in a still-born child with normal nose, lips and palate. Multiple associated defects include persistent left and right vena cava superior, patent foramen ovale and ductus arteriosus, and a pre-aortic communication in the azygos system. In addition to polydactyly and various muscular and visceral abnormalities, both right and left ulnar nerves run anterior to the medial epicondyle of the humerus.

Dr WALLS remarked on the thickness of the induseum griseum in spite of the presence of arhinencephaly, and suggested that the reason why some people could smell better than others depended on developmental and genetic factors. Dr MORTON mentioned a case of absence of the olfactory bulbs and tracts in an otherwise normal woman who could smell quite well in spite of their absence, so that presumably the olfactory nerves must in this case have found their way to the brain by some other route. In the foetus he had described he hesitated to say that the olfactory nerves were absent, though the bulbs certainly were so. Dr WALLS said he had seen a case where after traumatic damage to the olfactory bulbs the patient could still distinguish the presence of particulate matter in the air, though she had no true sense of smell.

Dr BRANDT questioned the suitability of the term arhinencephaly as applied to this case, since it seemed to him rather a case of multiple defects, such as were usually associated with defects of the olfactory apparatus.

Dr WHILLIS suggested that the choloboma was due to faulty rotation of the optic cup, and Dr DAVIES that it was due to a defect of the margin of a cup rather than of the foetal fissure. Dr MORTON answered that since the ocular muscles were normally developed any fault of rotation must have been corrected in later development, and that the extent of the retinal defects suggested a fault in the foetal fissure rather than in the margin of the cup.

G. E. H. FOXON and E. W. WALLS: 'Circulation through the heart and great vessels of the frog: a radiological study.'

Recent work by Foxon has thrown doubt upon the conventional description of the circulation through the heart and great vessels of the frog. In the hope of obtaining permanent records, injection experiments using radio-opaque contrast media (thorotrast, etc.) were made on pithed frogs (*Rana temporaria* and *R. esculenta*) and toads (*Bufo bufo*): the evidence obtained will be submitted. Briefly, it seems clear that mixing does take place in the ventricle, and that the present conception of the successive filling of the three divisions of the truncus arteriosus is not warranted by our results.

Dr FRANKLIN suggested that cineradiography would be helpful in following the spread of the injected material, and offered technical facilities for the work.

Professor YOFFEY remarked on the retrograde filling of the venous sinuses of the liver that appeared in some of the radiographs, and said that this might indicate the use of excessive pressure during the injection. Dr WALLS answered that the injection through one pulmonary vein had been controlled by a micrometer screw, and that the

application of too much pressure was indicated by the appearance of the opaque matter in the opposite pulmonary vein. Dr MORTON suggested the use of a reservoir to control the pressure, but Dr WALLS thought the viscosity of the opaque fluids would prevent the use of this method.

K. J. FRANKLIN: 'The height of the Eustachian valve correlated with the direction of the (right) anterior caval blood flow.'

Preamble. (i) A correlation, first advanced in the eighteenth century, between the presence of the valve and the absence of an intervenous tubercle, and vice versa, is belied by numerous exceptions. (ii) Cineradiographic records show streamlining of blood-flows within the right atrium (as elsewhere in the cardiovascular system).

Bases for the present correlation. (i) Cineradiographic records, taken during life in a number of species, of the course of the anterior caval blood-flow, and subsequent comparative examination of the relevant parts of the carefully fixed cadavers. (ii) Anatomical examination of the corresponding parts in representatives of all mammalian Orders. Total number of hearts examined, over 200.

Index of heart. This is defined as the maximum height of the Eustachian valve, expressed as a percentage of the heart length (anterior caval-atrial junction to apex).

Type of heart. Hearts are typed as follows:

I. Anterior caval flow impinges on caudal floor of right atrium just beyond Eustachian valve, and medial cornu of valve is attached to surface of fossa ovalis dorsal to sternal pillar of limbus fossae ovalis (as, e.g., in *Cavia porcellus* and *Dolichotis magellanica* specimens personally examined). Index 7 or over.

II. Same as Type I, except that medial cornu of valve is attached to sternal pillar of limbus fossae ovalis. Index 7 or over.

III. Anterior caval flow impinges on caudal floor of right atrium between Eustachian valve and right atrio-ventricular orifice. Index between 7 and 3.

IV. Anterior caval flow goes direct to right atrio-ventricular orifice. Index between 3 and 0.

The obvious outcome of the findings is that firstly one can state, from two post-mortem measurements, what was the direction of the (right) anterior caval blood-flow during life. Secondly, the index is a better expression than any absolute measurement of the height of the Eustachian valve. Thirdly, one is given a suggestion that this outgrowth is a result of functioning (bilateral stimulus from the posterior caval flow and a reflected component of the anterior caval flow), so one is less concerned with hypotheses about 'the use of this fugacious valve' (Jeffray, *Observations on the heart*, etc., 1835).

Professor CAVE complimented the author upon his successful application of the comparative method in his attempt at an interpretation of the nature of the Eustachian valve. Excellent as were the cardiac outline figures shown, familiarity with the actual specimens was even more convincing: his own examination of a representative series of mammalian hearts had provided corroboration of the hypotheses advanced by Dr FRANKLIN. The paper reflected the scientific value of careful direct observation in the field of gross morphology and was indeed Hunterian in its concept and execution.

Professor WEST said he had been puzzled by the description of the valve as a relic of the sinus valve, and was pleased to learn that it was an active structure with a reason for its existence. In man it varied widely in development; it might be a quarter or half an inch wide, solid or fenestrated or lace-like, muscular or fibrous in different individuals. It was a delightful idea that the valve arose as a resultant of two blood-flows, but he asked whether these flows were present throughout prenatal life, or if the valve altered at different stages of development as the direction of the flows changed.

In reply Dr FRANKLIN said that the relatively few human foetal hearts he had examined were mostly of Types II and III, with a few, probably, of Type IV, and there was, in this short series, no suggestion that the type changed towards full term, though this was, of course, a possibility in the case of any particular heart.

He suggested that up to a certain time in development, changes in direction of the foetal blood flows might lead to adhesions of part or whole of the valves, but that later on the results of such changes might be fenestration and atrophy. He knew of no explanation why one valve was muscular, another musculo-membranous and yet another membranous. He regretted that there was all too insufficient data in the literature about the valve, and no real knowledge of the blood flows in embryonic life, and he hoped that embryologists would be able, in the future, to fill in the gaps. Indeed, one of the main reasons for the communication was to pass on these lines of inquiry to the embryologists.

Professor KIRK said that one or two of Dr FRANKLIN'S figures recalled Frazer's observation that the Eustachian valve represented (*a*) a part of the right venous valve, and (*b*) a spur which develops on the wall of the right atrium between the foramen ovale and the opening of the coronary sinus, for the figures to which he referred, Professor KIRK suggested, showed a persistance of the spur after the disappearance of the right venous valve component.

Dr FRANKLIN replied that he did not know of this observation by Frazer, but that Shedenow (1943) had a somewhat comparable statement about the

development of the human venous valve. Professor KIRK's point strengthened the argument adduced in the communication, in that it reduced to 0 the already low indices given for one or two hearts of Type IV.

J. D. GREEN and G. W. HARRIS: 'A note on the blood supply and nerve supply of the hypophysis cerebri.'

Studies have been made of the blood supply and nerve supply of the hypophysis in a variety of animals. Slides will be shown to demonstrate some possible anatomical pathways by which the adenohypophysis is excited to secretion. The vascular link between the median eminence and pars distalis will be described, and the nerve supply of these vessels discussed.

Dr BAXTER said that in the adrenal there was no evidence of nervous control of the cortex, yet the amount of cortical substances secreted varied from time to time, so that a blood control was possible. He suggested that a similar control might be found in the hypophysis. Dr GREEN answered that nerve fibres were scarce in the hypophysis and agreed that blood control was probable. In the one gland adrenalin was known to stimulate the adrenal cortex, and a similar position might hold good for the pituitary.

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